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FURTHER DATA ON THE INHERITANCE OF BLUE IN POULTRY[1]

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I. PREVIOUS WORK

The principal facts concerning the genetic behavior of blue in the Andalusian breed of domestic fowl were presented in an earlier paper (Lippincott, 1918*a*). Previous work on the genetics of the blue Andalusian was reviewed and a limited number of further data were offered.

The latter showed that blue Andalusians are like black Andalusians in that they are self-colored. They are, on the other hand, like the blue-splashed Andalusians in that homologous pigmented feathers in both sexes have the same condition with reference to the restriction of pigment in the feather structure. The 1:2:1 ratio obtained from mating blue Andalusians together may be interpreted as the combination of two 3 to 1 ratios. These relationships are shown in Fig. 1.

The restriction of black pigment in the feather structure to give the blue appearance found in blue and in blue-splashed Andalusians was shown to be due to the action of a dominant factor *R*. The extension of black pigment to all feathers of the body as in both black and blue Andalusians, was found to be due to the action of another dominant factor *E*.

[1] Contribution from the Department of Genetics, Wisconsin Agricultural Experiment Station, No. 29, and from the Department of Poultry Husbandry, Kansas Agricultural Experiment Station, No. 15.

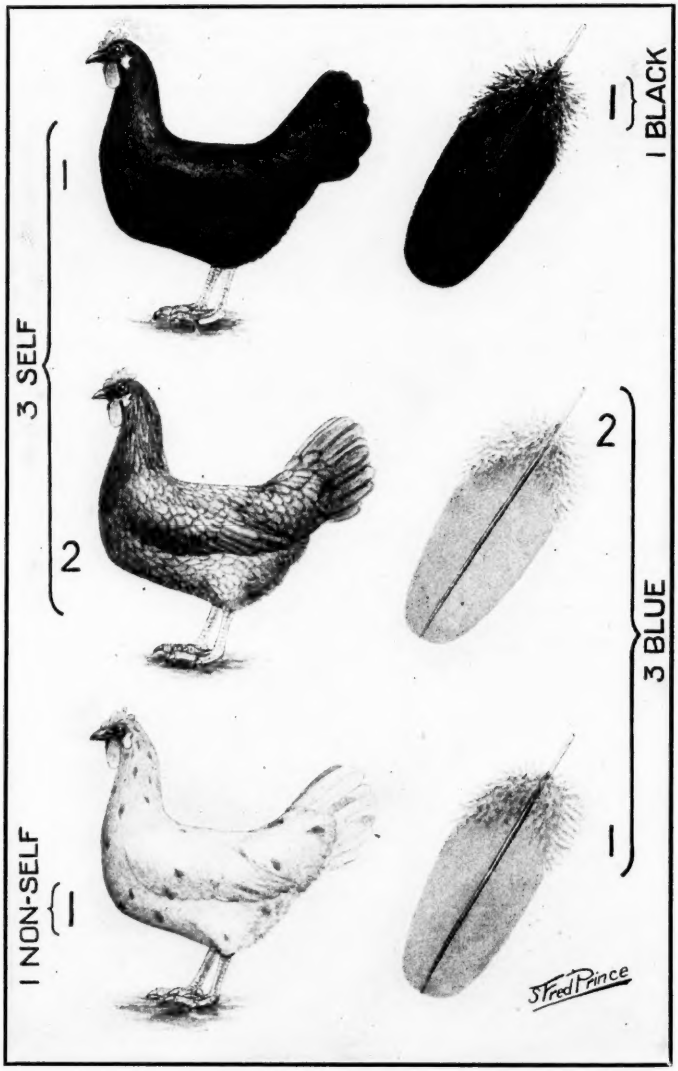


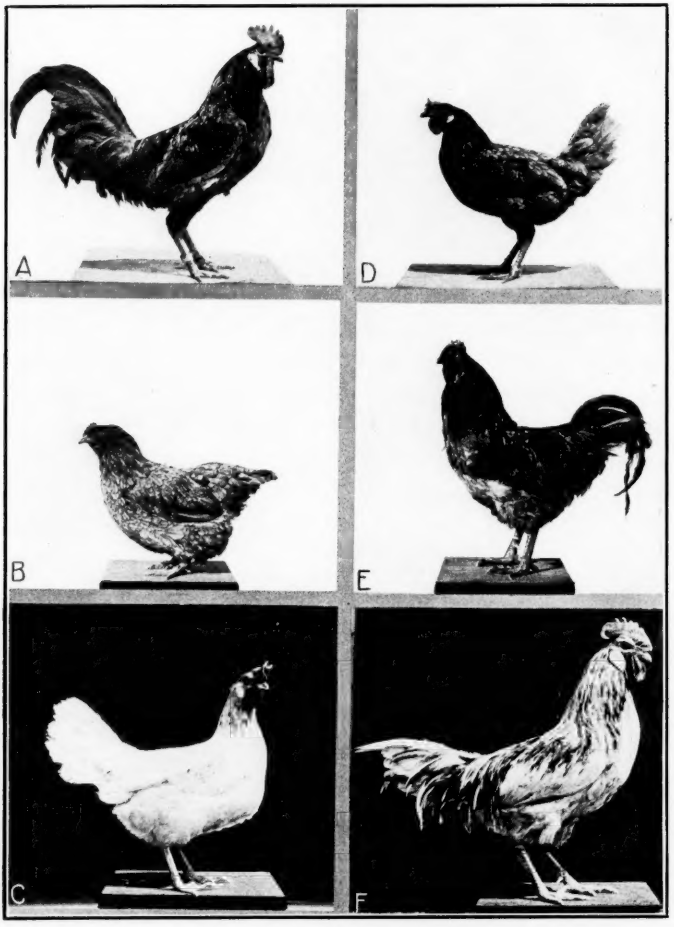
FIG. 1. Showing that the 1:2:1 ratio is a combination of two 3:1 ratios.

It was pointed out that while, on the basis of their expression in the phenotype it appeared more logical to consider these factors as dominants, each closely linked to the recessive allelomorph of the other, they may, so far as the experimental evidence shows, be considered as true allelomorphs occupying identical loci on homologous chromosomes, and each expressing itself independently of the other.

The finding of crossovers between *R* and *E* would be conclusive evidence proving the former of the two conditions proposed. It was shown that while no crossovers had been reported the critical data on the case were very limited and the likelihood of crossovers being detected and isolated by breeders is very small. It might well have been suggested further that even though crossing-over does rarely occur, for instance, so that less than one per cent. of the individuals are the product of crossover gametes, the chances of detecting them experimentally are small, considering the limited number of matings (as determined by the equipment available at most experimental institutions) which are likely to be devoted to a search for crossovers.

Though much has been made of the blue Andalusians as a "heterozygote phenotypically intermediate between the parental types" it was shown that while all self-blues so far found had proved to be heterozygous for *R* and *E*, they were not in the strict sense intermediate between the parental types. The F_1 progeny of a cross between blue-splashed Andalusians and white Wyandottes was reported as self-blue and far darker than either parent.

It was further shown in the earlier paper that *R* not only restricts black pigment, so as to render pigmented areas bluish-gray in appearance, but also affects the shape of the pigment granules, so that instead of appearing as rods as in black individuals, they are quite round. In this particular *R* is quite dominant over its allelomorph, whether one chooses to assume that the latter is *E* or *r*.



EXPLANATION OF PLATE I

The photographs shown in Plates I and II were taken by James Machir, my indebtedness to whom it is a pleasure to acknowledge.

FIG. A. Blue Andalusian male.
FIG. B. Blue Orpington female.
FIG. C. Blue-splashed Andalusian female.
FIG. D. Blue Andalusian female.
FIG. E. Blue Orpington male.
FIG. F. Blue-splashed Andalusian male.
A and *D*—Blue Andalusian.
B and *E*—Blue Orpington.
C and *F*—Splashed Andalusian.

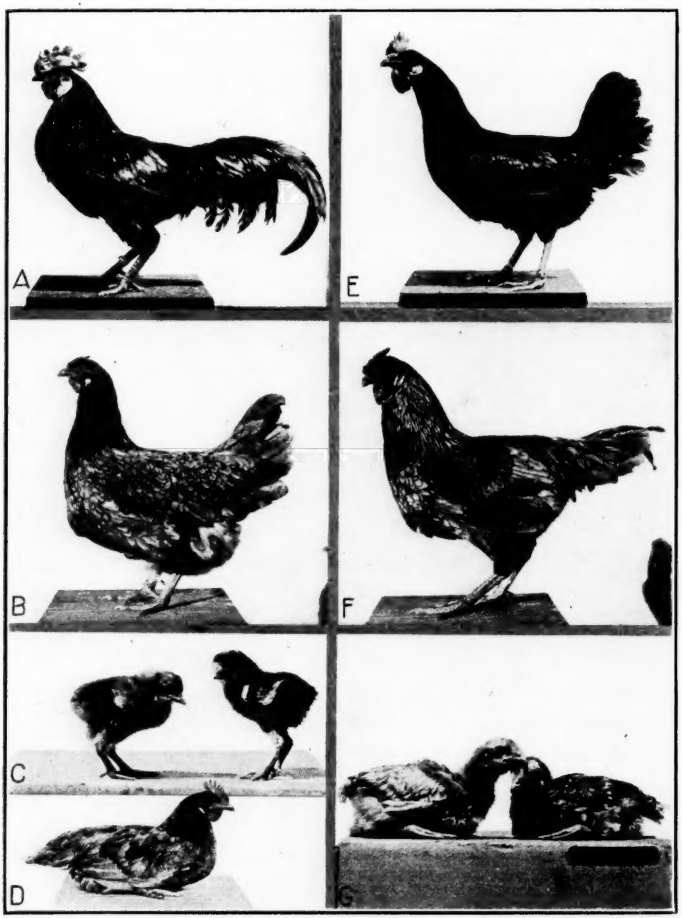
It was also shown that both the restricting and the rounding actions of *R* were interfered with in certain regions of both blue-splashed and blue males. In both color types the pigmented feathers of the neck (hackle), back, and saddle are black or bluish-black instead of blue as on the remainder of the body. The black pigment granules in these regions are for the most part rod-shaped rather than round. It was suggested that this interference with the action of *R* is a secondary sexual characteristic, presumably due to the presence of testicular or the absence of ovarian influence.

II. Purpose of the Present Paper

It is the purpose of this paper to present further data concerning the inheritance of blue and its relations to the sex glands, and to draw such conclusions as these data justify. A report is given of the breeding behavior of blue as found in the Andalusian, Orpington and Leghorn breeds, and of certain crosses of these breeds with each other and with other breeds, which do not possess blue varieties. The relations of the factors involved to certain factors present in the non-blue varieties of other breeds is considered and evidence concerning the relation of the sex-glands to the action of the factor *R* presented.

III. Material and Methods

The breeding stock used was from several sources, being in part from the pedigreed flock of the University of Wisconsin, where the work reported in the earlier paper was done. It was also in part from the pedigreed flock of Kansas State Agricultural College where the investigation was continued under the direction of Dr. Leon J. Cole of the University of Wisconsin, my indebtedness to whom it is a pleasure to acknowledge. The stock was, however, mostly from unpedigreed lines, though pure-bred within the meaning of the poultryman. In no case were individuals used which were not from families show-



EXPLANATION OF PLATE II

FIG. *A*. A black Andalusian male.

FIG. *B*. A blue F_1 female from a white Wyandotte × blue-splashed Andalusian cross.

FIG. *C*. A blue (at left) and a black (at right) chick in the down. These are offspring of a white Plymouth Rock ♂ × blue Andalusian ♀. The occipital spots inherited from the sire are plainly visible.

FIG. *D*. A young blue F_1 male from a blue-splashed × black Langshan cross.

FIG. *E*. A black Andalusian female.

FIG. *F*. A blue F_1 male from a white Wyandotte × blue-splashed Andalusian

ing the characteristics of their respective varieties with constancy in so far as could be learned. In as much as only varietal (color), as opposed to breed (shape) characteristics were being studied, less attention was paid to the latter in selecting material. In no case, however, were individuals used which showed disqualifying breed characteristics.

With a single exception no individual was used whose genotype proved to be inconsistent with the "breeding true" of the variety to which it belonged, or, in the case of the blue-splashed Andalusian, the variety from which it arose. This single exception was a blue-splashed Andalusian female (2107) purchased from a breeder who made only blue $\times$ blue matings. She proved to be heterozygous for *P*, a factor necessary for the production of black pigment. The family from which she arose must have been producing occasional whites which were, in all likelihood, being discarded as extremely light blue-splashed wasters from the blue $\times$ blue matings. This point was not followed up, however, and the facts ascertained. It has been by taking advantage of situations similar to this one that white varieties have been established in several breeds.

There were several individuals discovered whose factorial composition varied from the normal, or usual, for the varieties to which they respectively belonged. Owing to the particular factorial complex of which they were a part, however, these factors behaved as cryptomeres, not affecting the adult phenotype of the variety. Specific reference is made to these individuals in a later section of this paper.

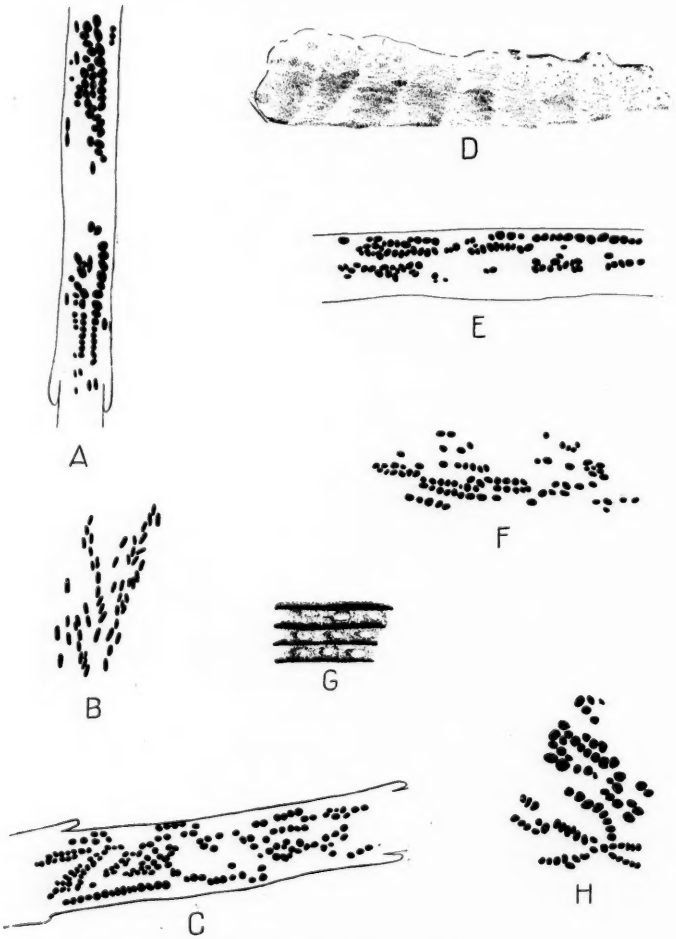
The matings were, for the most part, made in covered

cross. Indications of a factor or factors for lacing may be seen in the hackle and saddle feathers.

FIG. *G*. A blue-barred (at left) and a black-barred (at right) chick partly feathered. These are offspring of the same mating as the chicks shown in Fig. *C* this plate. The barring was inherited from their white Plymouth Rock sire.

A and *E*—Black Andalusian.

B and *F*—Blue F_1's.



EXPLANATION OF PLATE III

FIG. *A*. Appearance of pigment granules in a strand of down from a very dark blue crossbred chick. The sire was a blue F_1 from a white Wyandotte × blue-splashed Andalusian cross. The mother was a blue Andalusian. There are occasional rod-shaped granules. Camera lucida drawing.

FIG. *B*. Appearance of pigment granules in a definitive feather from a black Andalusian. Granules of the same shape are found in the down and definitive feathers of black Langshans, black Orpingtons and the black offspring from crosses with the several breeds used in this investigation. Camera lucida drawing.

FIG. *C*. Appearance of pigment granules in a strand of down from a blue

yards and every precaution taken to insure no mixing of matings and the proper identification of the eggs laid by each female in each mating. Not only was the assistant in charge of the trapnesting selected because of his habitual accuracy in details, but the eggs from each individual hen were kept together, separate from the eggs of other individuals, and carefully compared one with another before being put into the incubator. Any off type or off colored eggs were discarded, so far as these experiments are concerned. In spite of these precautions it is too much to hope that some errors have not crept in, though it is believed they are very few.

Owing to the fact that the original stock was of relatively unknown composition, it was necessary to make such matings as would not only throw light on the behavior of the factors under observation, but would also be likely to bring to light unsuspected factors whose action might interefere with the action of the genes being studied. This necessitated introducing test females in the matings where the males were uncertain, and of mating many of the females with test males a second season instead of repeating the mating already made. In both cases the result was to reduce greatly the numbers of offspring from some of the crucial types of matings, and considerable numbers of "test" offspring were hatched and described, for the reporting of which here there is no particular object.

The counts of living chicks were made in the down at hatching time and individual descriptions recorded, each

F_1 chick from a white Wyandotte × blue-splashed Andalusian cross. Camera lucida drawing.

FIG. *D*. Clumped appearance of pigment granules in a curved barbule from a definitive feather of a blue Andalusian.

FIG. *E*. Appearance of pigment granules in a strand of down from a blue F_1 chick from a blue-splashed Andalusian × white Plymouth Rock cross. Camera lucida drawing.

FIG. *F*. Appearance of pigment granules in a definitive feather from a blue Andalusian. Camera lucida drawing.

FIG. *G*. A small area of the web of a definitive feather from a black Andalusian. The cell boundaries and nuclei may be made out. There is no clumping of pigment within the cells.

FIG. *H*. Appearance of pigment granules in a definitive feather from a blue Andalusian. Camera lucida drawing.

chick being marked with a numbered wingband. The system of keeping pedigree records in use has been described elsewhere (Lippincott, 1918*b*) and need not be repeated. The descriptions were checked when the chicks were three weeks old, and again at some considerably later, though not specified, time, when the birds were leg-banded for the breeding-pen or laying-house, or were sent to market. The descriptions of all chicks dying were carefully checked at the time they were found, though a small number disappeared without their descriptions being checked. Unless there was reason to suspect that their classification might be likely to change after the taking of the first description such individuals were counted.

Fortunately the different classes of offspring could for the most part be distinguished in the down, and counts were accordingly made of chicks which reached an advanced stage of development but which failed to hatch. It was the practise to test all eggs for live germs at the end of the tenth day of incubation and remove all infertile and dead eggs. A second testing was made on the eighteenth day when all the dead eggs found were opened, the embryos described and their sex recorded. On the twenty-second day, after the hatch was well over, all the eggs which failed to hatch were opened and the descriptions of the dead chicks made a matter of record.

In most cases the embryos from crosses among the three color types of Andalusians which passed the first test, developed far enough so that the differentiation between color types could be made with precision after the down had been carefully washed, and dried with the aid of an electric fan. In those crosses involving recessive white parents, only those unhatched chicks could be counted which lived past the eighteenth day.

There were two possible sources of confusion in the classification of the chicks in the down. These were the differentiations between blacks and occasional very dark blues, and between blue-splashed and recessive whites.

The blacks and dark blues could quite readily be separated by examining the down of each chick microscopically. The blacks carry only rod-shaped pigment granules while in dark blue down rounded granules predominate. These are frequently arranged in rows as reported in my former paper (1918*a*, pp. 98, 99). In the case of every mating where this method of classification was brought into use to aid in distinguishing individuals which failed to hatch, down samples were saved at hatching time from the living dark blue and black chicks as well, and the first description, the record of the microscopic examination, and the later descriptions after definitive feathers were developed were carefully compared and checked. In nearly all cases the descriptions in the down and in the definitive feathers agreed.

In certain matings, however, it was found that descriptions in the down were not reliable and could not be counted. This was particularly true of one family of Andalusians which carried considerable red in the plumage and which has been referred to by Platt (1916) and Pearl (1917) in other connections. Within this family and its crosses the expression of the *R* factor in the heterozygote was frequently delayed so that individuals which were described in the down as blacks and which showed only rods under the microscope turned out to be blues when the definitive feathers appeared, and then showed the characteristic round pigment granules of the blue. None of the chicks tracing their ancestry to this family are included in the counts herein reported.

The possible source of confusion in the classification in the down of the blue-splashed and the white chicks arises from the fact that while the adults of the white Plymouth Rocks and white Wyandottes are pure white, or very slightly flecked with black, the chicks frequently carry considerable, though varying amounts of black pigment in the down, which gives certain regions a bluish appearance. This varies in degree from near black to slightly

smoky white. Fortunately for the problem in hand the localization of this pigment in the down of certain regions of the body is quite characteristic and quickly recognized. While a blue-splashed chick is frequently very light blue, as noted by Bateson and Punnett (1906, p. 20), the pigment is not localized on the top and back of the head, the wings in the region of the bow, and on the thighs, as it is on the potentially white chick, and the impression conveyed is very different. In potentially white chicks the remiges, which may be seen just starting to grow out from their follicles, are pinkish white and exhibit not the slightest trace of pigment. In the same feathers of the blue-splashed chick, on the other hand, there is a very noticeable bluish cast and usually at least one remex that is distinctly pigmented.

Though in pure-bred white Plymouth Rock and white Wyandotte chicks the pigment granules in the down are typically rod-shaped this fact is not of assistance in classifying with respect to white and blue-splashed offspring from crosses involving the factor *R*, since under its influence black pigment granules are round whether in a potentially white or a blue-splashed chick.

Not all chicks from pure-bred white Plymouth Rock and white Wyandotte matings exhibit this juvenile pigment. Some can only be recorded as white. It is of interest that the only chicks, three in number, which were originally described as "white, no pigment" or "creamy white" and later used in a breeding pen, have all proved to carry a factor for dominant white, as described in a later section of this paper. The number of such birds which have been tested is small and no general conclusions can be drawn, but the results are suggestive. It is rather interesting to note that a photograph of a group of white Plymouth Rock chicks in "The Plymouth Rock Standard and Breed Book" (American Poultry Association, 1919, p. 419), which is the official guide for the breeding and judging of all Plymouth Rocks, shows individuals

which are noticeably pigmented. In response to a letter of inquiry Professor Arthur Smith of the University of Minnesota, the editor of this book, tells me that my observation concerning the presence of pigment in these chicks is correct and he adds in substance that the pigmented chicks develop into the whitest adults.

The fertility and hatching power of the eggs from the various crosses here reported and the viability of the chicks hatched was increasingly disappointing from season to season. While the comparative coefficients of fertility and hatching power have not been calculated, the ratio between the eggs set and chicks hatched has undoubtedly been lower on the average, than for the pure-bred unrelated matings of the same and other breeds, set in the same incubator at the same time, and certainly lower than would be counted satisfactory in ordinary poultry husbandry practise.

The foregoing applies as well to the rate of mortality. As representative of the numbers surviving to grow definitive feathers in comparison with the counts recorded in the various tables, those of the F_2 from the blue-splashed Andalusian ♂ × white Wyandotte ♀ may be given. The counts made when the chicks were feathered were 47 blue, 18 blue-splashed, 37 black, and 42 white. The total count recorded (see Table IV, group 1) was 100 blue, 46 blue-splashed, 65 black, and 64 white. The reasons for the low hatchability and high mortality have not been established.

Until considerably more data than are now available have been secured it seems best to call attention to the possibility of crossing-over between the loci of *R* and *E* by indicating their possible recessive allelomorphs. It is accordingly the practise in this paper to indicate these factors thus: (*Re*) and (*rE*).

IV. The Relation of Phenotype to Sex

It is convenient to consider the relation of phenotype to sex before examining the progenies of the various

matings. In order to secure evidence concerning this relation, six blue Andalusian males were caponized during the summer of 1919. Into the body cavities of three of them ovarian tissue from nearly related females was introduced, the other three being kept as checks. The operating was done on July 24 and the birds turned out on range with hundreds of other birds one week later. On September 19 one of them (wingband 1387) was killed by a skunk. At that time it was entirely blue, there being less contrast between the regions that are dark in the male (hackle, back and saddle) and the other regions of the body than frequently appears in blue pullets before comb development indicates the approach of the first laying cycle, and indeed in many mature females. Although it was over four months old (hatching date, May 6, 1919) it appeared so much like an immature pullet that it was mistaken for one by the poultryman in charge and by the writer, until its record and description were consulted. Concerning the latter there did not seem to be any chance of error, since the scar made in opening the body cavity was plainly visible.

Such a situation indicates a fairly complete molt between July 24 and September 19. This is not surprising, however, since Rice, Nixon and Rogers (1908, p. 66) have shown that "from the incubator to the laying period the chicks experienced at least four molts, either partial or complete," and it is further well known that a close relation exists between molting and ovarian activity.

The other birds operated on at the same time were at once looked up and described. One of them (wingband 1855) was found to be somewhat intermediate in condition, some of the feathers of the neck and saddle being blue, but somewhat darker in shade than the normally (in the male) blue regions of the body. There were, however, a few scattered feathers which were almost black from the tip halfway down the web toward the fluff. About midway between the tip of the feather and the be-

ginning of the fluff there was a distinct line of demarcation where the black or near-black became a distinct blue. This chick was hatched a little over two weeks later (May 22) than 1387 and had apparently not gone through a complete molt, some feathers in process of growth at the time of the operation and showing ovarian influence on the last regions to develop still remaining.

On October 26 this bird was killed, apparently by a rat, at which time all of the feathers of the neck and saddle regions were distinctly blue, though considerably darker than other parts of the body. The shape of the feathers was characteristically female.

The third male into which ovarian tissue was introduced (wingband 1480) showed no influence of the introduced tissue on September 19. This condition still prevailed when it was sent to market October 26. It appeared normal for a blue capon of that age, over five months, the hackle and saddle being very dark and characteristically male in shape. Presumably the ovarian tissue introduced atrophied without having any effect.

Of the cockerels which were caponized, but had no ovarian tissue introduced, one (wingband 1859) died soon after the operation. The other two (wingbands 1415 and 1492) showed and continued to show typical blue capon characteristics with regard to the color and shape of the saddle and hackle feathers. The feathers were fully as dark as in normal males of the same age, and as they matured were even longer than their homologs in normal males. This result is precisely the same as that observed by the writer several times in blue capons, concerning which no descriptive records were kept.

In this connection it should be observed that in the family of Andalusians here dealt with, it has been not infrequently noticed that certain nearly grown pullets whose combs have not begun to develop, show only very dark feathers in the regions of the neck and back. These same birds after their combs begin to redden, thereby

indicating ovarian activity and the onset of laying, appear to pass through a molt or partial molt whereby the dark feathers of the back region particularly, are gradually replaced by those of a clearer blue. The necks of such females usually remain dark, showing considerable contrast with the other regions of the body, though being by no means as dark as the same region of the blue male.

Although the number of desexed males into which ovaries were introduced was small, it seems fair to conclude in the light of the evidence concerning testicular (Goodale, 1916) and ovarian (Goodale, 1918; Cole and Lippincott, 1919) influence in fowls that the failure of the factor *R* to express itself as fully in the neck, back and saddle regions of the blue and blue-splashed males as in the females is due to the lack of some necessary cooperative action on the part of the ovary, and not to any inhibitive action on the part of the testis.

V. The Breeding Behavior of Andalusians

New data concerning the breeding behavior of the three color types of Andalusians, as shown by several types of matings, are presented in Table I.

TABLE I

SHOWING THE NUMBERS AND COLOR TYPES OF PROGENIES FROM VARIOUS ANDALUSIAN CROSSES[2]

Group	♂♂	♀♀		Blue-spl. (*Re*) (*Re*)	Blue (*Re*) (*rE*)	Black (*rE*) (*rE*)
1..	Blue	× blue	Obtained...	46	104	64
	(*Re*) (*rE*)	(*Re*) (*rE*)	Theoretical.	53.5	107	53.5
2..	Blue	× black	Obtained...	00	25	24
	(*Re*) (*rE*)	(*rE*) (*rE*)	Theoretical.	00	24.5	24.5
3..	Black	× blue	Obtained...	00	113	90
	(*rE*) (*rE*)	(*Re*) (*rE*)	Theoretical.	00	101.5	101.5
4..	Blue	× blue-splashed	Obtained...	1	1	0
	(*Re*) (*rE*)	(*Re*) (*Re*)	Theoretical.	1	1	0
5..	Blue-splashed	× blue	Obtained...	35	33	0
	(*Re*) (*Re*)	(*Re*) (*rE*)	Theoretical.	34	34	0
6..	Black	× blue-splashed	Obtained...	0	138	0
	(*rE*) (*rE*)	(*Re*) (*Re*)	Theoretical.	0	138	0
7..	Blue-splashed	× black	Obtained...	0	56	0
	(*Re*) (*Re*)	(*rE*) (*rE*)	Theoretical.	0	56	0
8..	Blue-splashed	× blue-splashed	Obtained...	0	12	0
	(*Re*) (*Re*)	(*Re*) (*Re*)	Theoretical.	0	12	0

[2] Andalusians are normally homozygous for *P*, a factor necessary for the production of black pigment.

These results are in substantial accord with those of Bateson and Punnett (1906, p. 20). A somewhat marked departure from the theoretical expectation appears in group (3) of black ♂♂ × blue ♀♀ matings, the agreement in the reciprocal cross (group 2) being as close as possible. This departure from expectation is due to the progeny of a single pair of birds (♂136M and ♀2005) which produced 25 blues and 6 blacks. If the latter are left out of consideration the results are 88 blues and 84 blacks.

However, even in the case of the progeny of ♂136M and ♀2005 the Dev./P.E. = 4.1, which indicates a deviation of doubtful significance. The results of this mating were carefully considered from the standpoint of crossing-over, but there is no indication of its having occurred.

According to these results the genetic compositions of the three color types of Andalusians used in these experiments were as follows: blue-splashed = $(Re)(Re)$, blue = $(Re)(rE)$, and black = $(rE)(rE)$. There was no evidence of crossing-over between R and E having occurred.

VI. Data from Crosses of Andalusians with Certain Recessive White Breeds

In the previous paper (1918*a*, p. 106) the writer reported a small number of data on a cross between a white Wyandotte ♂ and a blue-splashed Andalusian ♀. These have been considerably increased in amount and the reciprocal cross made. Further, both blue and black Andalusians have been crossed reciprocally with white Wyandottes and all three Andalusian color types crossed reciprocally with white Plymouth Rocks. The data from these several matings are set forth in Table II.

The crosses were made in the twelve possible ways, from eleven of which offspring were secured, the one type of mating which failed to produce offspring being the white Wyandotte ♂ × black Andalusian ♀. Inasmuch as

there is no evidence that any of the factors here under observation are sex-linked and there is considerable evidence that they are not, this omission is not serious.

TABLE II

SHOWING THE RESULTS OF CROSSING THE THREE-COLOR TYPES OF ANDALUSIANS WITH WHITE WYANDOTTES AND WHITE PLYMOUTH ROCKS

Group	♂♂	♀♀		Blue	Black
1.	Blue-splashed Andalusian *PP(Re)(Re)*	× white Wyandotte *pp(rE)(rE)*	Obtained	65	00
			Theoretical	65	00
2.	White Wyandotte *pp(rE)(rE)*	× blue-splashed Andalusian *PP(Re)(Re)*	Obtained	50	00
			Theoretical	50	00
3.	Blue-splashed Andalusian *PP(Re)(Re)*	× white Plymouth Rock *pp(rE)(rE)*	Obtained	179	00
			Theoretical	179	00
4.	White Plymouth Rock *pp(rE)(rE)*	× blue-splashed Andalusian *PP(Re)(Re)*	Obtained	87	00
			Theoretical	87	00
5.	Blue Andalusian *PP(Re)(rE)*	× white Wyandotte *pp(rE)(rE)*	Obtained	27	24
			Theoretical	25.5	25.5
6.	White Wyandotte *pp(rE)(rE)*	× blue Andalusian *PP(Re)(rE)*	Obtained	13	18
			Theoretical	15.5	15.5
7.	Blue Andalusian *PP(Re)(rE)*	× white Plymouth Rock *pp(rE)(rE)*	Obtained	80	55
			Theoretical	67.5	67.5
8.	White Plymouth Rock *pp(rE)(rE)*	× blue Andalusian *PP(Re)(rE)*	Obtained	24	32
			Theoretical	28	28
9.	Black Andalusian *PP(rE)(rE)*	× white Wyandotte *pp(rE)(rE)*	Obtained	00	18
			Theoretical	00	18
10.	Black Andalusian *PP(rE)(rE)*	× white Plymouth Rock *pp(rE)(rE)*	Obtained	00	132
			Theoretical	00	132
11.	White Plymouth Rock *pp(rE)(rE)*	× black Andalusian *PP(rE)(rE)*	Obtained	00	28
			Theoretical	00	28

The results of these crosses are understandable on the assumption suggested in the earlier paper that the individuals from the recessive white races are homozygous for the factors *E* and *p*, *p* being the recessive allelomorph of *P*, a factor necessary for the production of black pigment in the feathers. Sturtevant (1912) first suggested that Wyandotte white is recessive, a fact which was overlooked in my earlier paper. Morgan and Goodale (1912, p. 115) have made a similar assumption for the white Plymouth Rock.

Since in the series of experiments being reported here, reciprocal crosses of white Wyandottes and white Plymouth Rocks gave only whites, thereby showing no

evidence of recombination, it seems fair to assume that the white of both breeds is due to the same recessive factor *p* in homozygous condition.

The condition of the white Rocks and white Wyandottes reported in Table II, with reference to *E*, appears clear, since in all crosses with blue-splashed Andalusians (and as will appear later, in the case of the Wyandotte, with blue-splashed Orpingtons) which are homozygous for *P* and *R*, but do not carry *E*, all offspring, 381 in number, were without an exception, blue (see mating groups 1 to 4, Table II).

On this basis blue Andalusians, *PP*(*Re*)(*rE*), mated with such recessive whites should produce blues and blacks in equal numbers. Mating groups 5 to 8, inclusive, in Table II show the results of such matings, which combined give 144 blues to 129 black (136.5 to 136.5 would be equality), a fair realization of the expectation.

As would be expected from the foregoing, crosses of similar recessive whites with black Andalusians (*PP*(*rE*)(*rE*)) (see Table II, groups 9 to 11, inclusive) gave only blacks. Of these there were in all 178 individuals and no exceptions.

The offspring of the crosses reported in Table II frequently gave evidence that the recessive white parents carried pattern factors as cryptomeres, but for the sake of clearness these complications, which have nothing directly to do with the study in hand, have been ignored in summarizing the data. As was to be expected, the white Plymouth Rocks carried the sex-linked pattern factor for barring. All pigmented offspring by a white Rock sire showed evidences of barring as soon as the definitive feathers appeared. Two such, the offspring of a white Plymouth Rock ♂ and blue Andalusian ♀ are shown in Fig. *G*, Plate II. Even at hatching, the occipital spot, which may be a juvenile effect of the factor for barring, gave notice of the presence of the barring factor. In the work here reported it was found possible to classify

in the down pigmented offspring of a non-barred ♂ × white Plymouth Rock ♀ cross accurately with regard to sex, by the presence or absence of the occipital spot. Morgan and Goodale (1912) made use of this spot in classifying barred and non-barred chicks which failed to hatch and Punnett (1919) also has made use of it in sorting newly hatched cross-bred chicks according to sex.

The progeny of crosses involving white Wyandottes frequently displayed Wyandotte lacing of a lesser or greater degree of perfection, though the appearance of this pattern was neither as constant nor as distinct as that of the barred pattern. The appearance of the lacing was to be expected if, as is generally stated in the literature on Wyandottes (see McGrew, 1901), the white variety was derived directly from the silver Wyandotte, which is laced.

In connection with these recessive white crosses is to be noted the fact that several white individuals, although "pure-bred" in the terminology of the poultryman, gave results which differed from the foregoing. Four white Wyandotte females proved to carry both the *R* and *E* factors and were of the same composition with respect to these factors as a pure-bred blue Andalusian, but unlike the blue Andalusian they carried *p* in the homozygous condition. One of these, which has already been reported on elsewhere (Lippincott, 1919), carried the sex-linked pattern factor for barring as well. Dryden (1916, p. 67) has also reported a white Wyandotte carrying a factor for barring.

One white Plymouth Rock and eight white Wyandottes proved to be heterozygous for a factor for dominant white. These were tested and found to be homozygous for *p*. In other words they carried both dominant and recessive white. Bateson and Punnett (1905, p. 117) appear to have had birds of this type and Dryden (1916, p. 66) reports a white Wyandotte which produced only white chicks when mated to a black Minorca, hence must

have been homozygous for a dominant white factor. Whether it carried *P* or *p*, the evidence does not show.

So far no attempt has been made to ascertain whether this factor for dominant white is the same as that normally carried by the white Leghorn and which Hadley (1913 and 1914) designated as *I*. For convenience and to recognize the possibility of its differing from *I* the factor here dealt with is referred to in this paper as I^P (inhibitor of pigment) and its allelomorph as i^P.

VII. Back-crosses of F_1's from Blue-splashed Andalusian × Recessive White Matings

The results of crossing the F_1 blues from the blue-splashed Andalusian × recessive white crosses is shown in Table III.

While by no means all possible back-crosses have been made, enough are represented to show clearly that factors *R* and *E* were appearing in approximately equal numbers, and that this was also true of *P* and *p*, though in some cases the presence of I^P complicated matters somewhat. It was, unfortunately, not always possible to use the actual parents in making back-crosses and though individuals from the same families were employed, this proved to be no criterion that they would be of the same genotype as the individuals used in the original cross. There can be no question as to their factorial composition, however, as each individual has been either deliberately tested or had happened to be so mated for another purpose as to give dependable evidence on its composition with respect to I^P and *p*.

So far as it goes, the evidence, which is substantiated by the results of other crosses to be reported in a later section of this paper, also shows that the meeting of *P* and *R* was according to chance, thereby indicating no linkage between these two factors.

It will be noted that the blue F_1 ♀♀ in group 5 of Table III had a blue Andalusian mother instead of a blue-

TABLE III

SHOWING THE RESULTS OF BACK-CROSSING F_1 BLUES FROM BLUE AND BLUE-SPLASHED ANDALUSIAN × RECESSIVE WHITE CROSSES

Group	♂♂	♀♀		Blue-Splashed	Blue	Black	White
1...	Blue F_1 $\frac{\text{spl. And. } ♂^3}{\text{wh. Wyand. } ♀}$ $Pp(Re)(rE)$	× blue-splashed Andalusian $PP(Re)(Re)$	Obtained	15	12	00	00
			Theoretical	13.5	13.5	00	00
2...	Blue F_1 $\frac{\text{wh. Wyand. } ♂}{\text{spl. And. } ♀}$ $Pp(Re)(rE)$	× blue-splashed Andalusian $PP(Re)(Re)$	Obtained	4	3	00	00
			Theoretical	3.5	3.5	00	00
3...	Blue F_1 $\frac{\text{spl. And. } ♂}{\text{wh. Rock } ♀}$ $Pp(Re)(rE)$	× blue-splashed Andalusian $PP(Re)(Re)$	Obtained	25	24	00	00
			Theoretical	24.5	24.5	00	00
4...	Blue F_1 $\frac{\text{wh. Wyand. } ♂}{\text{spl. And. } ♀}$ $PpiP_iP(Re)(rE)$	× white Wyandotte $ppIP_iP(rE)(rE)$	Obtained	00	12	7	64
			Theoretical	00	10.375	10.375	62.250
5...	White Plymouth Rock $pp(rE)(rE)$	× blue F_1 $\frac{\text{wh. Wyand. } ♂}{\text{blue And. } ♀}$ $Pp(Re)(rE)$	Obtained	00	8	8	24
			Theoretical	00	10	10	20
6...	White Wyandotte $ppIP_iP(rE)(rE)$	× blue F_1 $\frac{\text{spl. And. } ♂}{\text{wh. Wyand. } ♀}$ $PpiP_iP(Re)(rE)$	Obtained	00	3	7	33
			Theoretical	00	5.375	5.375	31.250

[3] This convention in this and subsequent tables is used to indicate the kind and direction of the original cross.

splashed. From the nature of the behavior of the factors *R* and *E* already described, this would make no difference with regard to the blue offspring, for the blue progeny of a blue Andalusian female by a white Wyandotte male would be of exactly the same composition with respect to *R*, *E*, and *P* as *all* the offspring of a blue-splashed Andalusian mother by the same sire.

It will also be noted in this group (5) that while the father of the F_1 blue was a white Wyandotte, the male used in this cross was a white Plymouth Rock. Since it has been shown that for the factors being studied, white Plymouth Rocks and white Wyandottes are identical, this should not affect the ratios.

VIII. The F_2 Ratios from Blue-splashed Andalusian × Recessive White Matings

The F_2 ratios from various blue-splashed Andalusian × recessive white crosses are shown in Table IV.

As will be seen, the four F_2 classes predicted for such crosses in the writer's earlier paper (1918*a*, p. 113) on the basis of the F_1 results, have been obtained. No other classes have appeared. This would seem to indicate that the factorial compositions of the blue-splashed Andalusians and white Wyandottes then proposed were correct and that the white Plymouth Rocks used were of the same composition with respect to the factors *R*, *E* and *P* as were the white Wyandottes.

Seven F_1 blue males were used in securing the F_2 ratios. The legband numbers of these males may be found in Table IV, in the column headed "Band No." The direction of the original cross is indicated for each male and for the group of females with which he was mated. The direction of the cross was the same for the males and the females in all cases but two. Males 296M and 258M were mated with females which were products of the same crosses, respectively, as they themselves (groups 2 and 7), and also with females from the reciprocal crosses (groups 3 and 8).

TABLE IV

SHOWING F_2 RATIOS FROM CROSSES OF BLUE-SPLASHED ANDALUSIANS AND WHITE WYANDOTTES AND WHITE PLYMOUTH ROCKS[4]

Group	♂	Band No.	♀♀		Blue	Blue-spl.	Black	White
1...	Blue F_1 spl. And. ♂ / wh. Wyand. ♀	86E ×	blue F_1 spl. And. ♂ / wh. Wyand. ♀	Obtained	100	46	65	64
				Theoretical	103.1250	51.5625	51.5625	68.7500
	$X^2 = 4.5747$		$P = .2099$					
2...	Blue F_1 spl. And. ♂ / wh. Wyand. ♀	296M ×	blue F_1 spl. And. ♂ / wh. Wyand. ♀	Obtained	73	24	39	48
				Theoretical	69	34.5	34.5	46
	$X^2 = 4.0998$		$P = .252516$					
3...	Blue F_1 spl. And. ♂ / wh. Wyand. ♀	296M ×	blue F_1 wh. Wyand. ♂ / spl. And. ♀	Obtained	45	17	20	27
				Theoretical	40.875	20.4375	20.4375	27.2500
	$X^2 = .8759$		$P = .83592$					
4...	Blue F_1 wh. Wyand. ♂ / spl. And. ♀	66M ×	blue F_1 wh. Wyand. ♂ / spl. And. ♀	Obtained	19	14	7	6
				Theoretical	17.25	8.625	8.625	11.5
	$X^2 = 5.7301$		$P = .1278$					
5...	Blue F_1 wh. Wyand. ♂ / spl. And. ♀	65E ×	blue F_1 wh. Wyand. ♂ / spl. And. ♀	Obtained	94	41	44	42
				Theoretical	82.875	41.4375	41.4375	55.2500
	$X^2 = 4.6871$		$P = .1998$					
6...	Blue F_1 spl. And. ♂ / wh. Rock ♀	46E ×	blue F_1 spl. And. ♂ / wh. Rock ♀	Obtained	34	10	17	18
				Theoretical	29.625	14.8125	14.8125	19.75
	$X^2 = 2.7349$		$P = .4395$					
7...	Blue F_1 spl. And. ♂ / wh. Rock ♀	258M ×	blue F_1 spl. And. ♂ / wh. Rock ♀	Obtained	51	26	39	51
				Theoretical	62.625	31.3125	31.3125	41.75
	$X^2 = 6.7510$		$P = .081786$					
8...	Blue F_1 spl. And. ♂ / wh. Rock ♀	258M ×	blue F_1 wh. Rock ♂ / spl. And. ♀	Obtained	14	3	3	1
				Theoretical	7.8750	3.9375	3.9375	5.25
	$X^2 = 8.20$		$P = .042668$					
	Total ratios for all crosses $X^2 = 8.6410$ $P = .0353$			Obtained	430	181	234	257
				Theoretical	413.25	206.625	206.625	275.500

[4] The formulæ of color types involved in these crosses are: blue-splashed Andalusian *PP* (*Re*) (*Re*), white Rocks and Wyandottes *pp*(*rE*)(*rE*), and F_1 blues *Pp*(*Re*)(*rE*).

As may be seen by inspection of Table IV, but one male (296M) gave a group of offspring (3) which was very close to expectation. The chances that as great a deviation as this one would appear as a result of random sampling are four to one. The mothers of this group were the product of a cross which was the reciprocal of that which produced their sire. The offspring of 296M when mated with females which were the product of the same cross as himself (group 2) gave a deviation so great that the chances against its appearing as a result of random sampling are three to one. The chances of the appearance of deviations as great as those shown by the offspring groups of the other males were as follows: 86E (group 1) one chance in a little less than five; 66M (group 4) one chance in about eight; 65E (group 5) one chance in approximately five; 46E (group 6) one chance in about two and a quarter; 258M (group 7) once in about twelve times with females from the same cross as he, and once in twenty-five when mated with females from a reciprocal cross (group 8).

It would be unusual, though not impossible, to have so many comparatively wide deviations from expectation simply as a result of random sampling.

If the genetic constitution of the F_1's was as has been previously postulated, and these were in fact all chance deviations, it would be highly probable that the lumping of all the data given in Table IV would approximate the calculated ratio fairly closely.

The lumped data are given at the bottom of Table IV. It will at once be seen that the goodness of fit as measured by P is poorer than the poorest constituent group, and would be probable, on the basis of random sampling, once in about twenty-eight times. It seems fairly clear that some disturbing force was operative.

The two possible causes of disturbance which present themselves are linkage and a differential viability of classes, or it might be a combination of the two.

Linkage between the two principal pairs of factors involved in the crosses, Pp and $(Re)(rE)$, may not be appealed to because the only possible linkage relation would produce results diametrically opposed to those with which we are confronted. Since according to our hypothesis the recessive white parents were in each case of the composition $pp(rE)(rE)$ and the blue-splashed Andalusian parent $PP(Re)(Re)$, it is evident that linkage would require the production of $p(rE)$ gametes by the F_1 blues, more often than $P(rE)$ gametes. And similarly the combination $P(Re)$ should also appear more often than $p(Re)$.

A complete linkage between these pairs of allelomorphs would result in an F_2 ratio of 1 blue-splashed and 2 blue to 1 white, the blacks not appearing. The tendency of even weak linkage would be to reduce the proportional number of blacks. This should be true irrespective of the direction of the cross. It would further be true, that unless crossing-over occurred in both sexes any linkage whatsoever would inhibit the production of F_2 blacks homozygous for P. As will be shown in a later section of this paper, however, F_2 blacks homozygous for P have been identified. Even a casual inspection of Table IV shows that a relative preponderance of blacks is a quite constant characteristic.

Crossing-over in the male fowl has been found by Goodale (1917) and in the male pigeon by Cole and Kelley (1919). The latter investigators definitely state that there is no crossing-over of sex-linked factors in the female pigeon. Goodale states that none had been observed in the female fowl, but that a definite test of the matter would be made later. So far as the writer is aware no further report has been made. It should perhaps be pointed out that so far only sex-linked factors have been dealt with, no autosomal linked groups in birds having so far been reported.

There are no F_2 data available from crosses where p and (Re) are found in one parent and P and (rE) in the

other. The F_1's from such a cross have been secured by mating an extracted white of the composition $pp(Re)(Re)$ with a black Andalusian, $PP(rE)(rE)$, which gave all blues. From these an attempt will be made to secure F_2's in considerable numbers. Back-crosses to the parental types will also be made. The F_2's should approximate the same ratios as appear in Table IV and also give some evidence on the second possible explanation of the persistent deviations about to be discussed.

The calculation of theoretical expectancies presupposes the equal viability of all phenotypic and genotypic classes. If for any reason the individuals of one or more of the obtained classes tend to be less viable than certain other classes, deviation from expectancy will occur if the lack of viability expresses itself prior to making the counts.

As has already been pointed out, the lumping of the data presented in Table IV brings forth a poorer fit than is shown in any of the constituent groups. The deficient classes are the blue-splashed and the white, while the most preponderant class relatively is the black.

It seems to be a rather tacit assumption among poultrymen, particularly, it must in truth be said, among those breeding pigmented varieties, that the recessive white varieties are less vigorous (and so in all probability less viable) than the pigmented varieties of the same breeds. In how far this assumption is based on fact there is no critical evidence to call upon.

Regarding the relative viability of splashed and self-colored races there is no suggestion from any source. Splashed varieties are, so far as I am aware, nowhere bred as such, and the experience of practical breeders may accordingly not be appealed to.

While in the case in hand the assumption of low viability on the part of the individuals of the splashed and recessive white classes seems to correspond with the facts, such an assumption, though convenient, is not cor-

roborated by other evidence. That the splashed classes are not necessarily always deficient is shown by the progeny of the blue-splashed × blue mating in Table I, group 5, and of the F_1 blue × blue-splashed matings in Table III, groups 1, 2 and 3.

The latter fact suggests that possibly certain individuals used in these matings carried recessive factors tending to cause low viability, which were linked to the factor *R*. Until the fact of a differential viability is demonstrated, however, it is useless to speculate on this possibility. The reason for the deficiencies in the blue-splashed and also in the white classes, therefore can not at present be determined.

IX. Identification of the F_2 Genotypes

As indicated in my former paper (1918*a*, p. 113) the genotypes expected in the several F_2 phenotypes from the blue-splashed × recessive white crosses are as follows: blue, *PP*(*Re*)(*rE*) and *Pp*(*Re*)(*rE*); blue-splashed, *PP*(*Re*)(*Re*) and *Pp*(*Re*)(*Re*); black, *PP*(*rE*)(*rE*) and *Pp*(*rE*)(*rE*); white, *pp*(*Re*)(*Re*), *pp*(*Re*)(*rE*) and *pp*(*rE*)(*rE*). Although the limitations of equipment were such that comparatively few F_2 individuals could be tested, fortunately all of the genotypes but one have been identified by making the appropriate crosses. The blues mated to individuals homozygous for *p* and *E* gave blues and blacks in equal numbers, or, blues, blacks and whites in the approximate ratio of 1:1:2, as the case might be. The blue-splashed mated to individuals of the same constitution produced all blues, or, equal numbers of blues and whites, depending upon whether or not they were homozygous with respect to *P*. Similarly the blacks gave all blacks, or, blacks and whites, depending upon their condition with respect to *P*.

The whites on the other hand were mated to blacks known to be homozygous for *P* and *E*. The *pp*(*Re*)(*Re*) whites, as mentioned in an earlier section of this paper,

gave all blues, just as would blue-splashed Andalusians. The *pp*(*Re*)(*rE*) whites produced blacks and blues in approximately equal numbers, exactly as would blue Andalusians. The parental white genotype *pp*(*rE*)(*rE*), which would give all blacks, was curiously enough, the one of the whites which did not happen to be selected for testing.

It is important to note that while eight out of the nine F_2 genotypes were identified, no genotypes were found other than those expected.

X. Data on Andalusian × Black Langshan Crosses

It appeared desirable, in order to ascertain whether there was anything inherent in Andalusian black which made its relation to Andalusian blue different from that of other black breeds, to make certain matings of Andalusians with black Langshans. The Langshan was chosen because not only is it a different breed, but it also belongs to a different group of breeds. The original black Langshans were, according to Brown (1906, p. 63), imported from China, while the Andalusians, according to the same authority (p. 107), originated from native stocks along the borders of the Mediterranean Sea. So far as is known they have nothing in common in their immediate ancestry. Davenport (1914) even points to the probability that the immediate wild ancestors of the Asiatic breeds differed from those of the Mediterranean breeds. If blacks differ in their relation to Andalusian blue it would seem probable that Andalusian black and Langshan black might show this difference.

The results of the Andalusian-Langshan matings are shown in Table V. As may be seen readily by reference to this table the results are in every case precisely those which might be expected if a black Andalusian had been substituted for the black Langshan. So far as the principal factors under discussion are concerned it appears that the black Langshans used were identical in composi-

TABLE V

SHOWING THE RESULTS OF SEVERAL ANDALUSIAN × BLACK LANGSHAN CROSSES

Group	♂	♀♀		Blue Splashed	Blue	Black
1..	Blue Andalusian	× black Langshan	Obtained	0	34	31
	PP(Re)(rE)	*PP(rE)(rE)*	Theoretical	0	32.50	32.50
2..	Blue Andalusian	× black wh. Wyand. ♂ / black Lang. ♀	Obtained	0	15	21
	PP(Re)(rE)	*Pp(rE)(rE)*	Theoretical	0	18	18
3..	Blue-splashed Andalusian	× black Langshan	Obtained	0	65	0
	PP(Re)(Re)	*PP(rE)(rE)*	Theoretical	0	65	0
4..	Black Andalusian	× black Langshan	Obtained	0	0	11
	PP(rE)(rE)	*PP(rE)(rE)*	Theoretical	0	0	11
5..	Blue blue And. ♂ / black Lang. ♀	× blue blue And. ♂ / black Lang. ♀	Obtained	4	9	5
	PP(Re)(rE)	*PP(Re)(rE)*	Theoretical	4.5	9	4.5
6..	Blue blue And. ♂ / black Lang. ♀	× black blue And. ♂ / black Lang. ♀	Obtained	0	12	9
	PP(Re)(rE)	*PP(rE)(rE)*	Theoretical	0	10.5	10.5
7..	Blue blue And. ♂ / black Lang. ♀	× black Langshan	Obtained	0	12	13
	PP(Re)(rE)	*PP(rE)(rE)*	Theoretical	0	12.5	12.5
8..	Blue blue And. ♂ / black Lang. ♀	× blue Andalusian	Obtained	5	6	2
	PP(Re)(rE)	*PP(Re)(rE)*	Theoretical	3.25	6.5	3.25

tion with the black Andalusians, being *PP(rE)(rE)*. The condition of the Langshan with respect to *P* was found by mating individuals with white Wyandottes, whereby only black, *i.e.*, pigmented, offspring were produced.

XI. THE RELATION OF ORPINGTON BLUE TO ANDALUSIAN BLUE

Among the Orpingtons, an English breed, is a blue variety. Like the blue Andalusian it is an inconstant breeder with regard to color, segregating into blue-splashed and blacks as well as blues. Though by no means as widely bred as the blue Andalusians, it has numerous admirers, some of whom have claimed verbally to the writer that the proportion of wasters, *i.e.*, blue-splashed and blacks, was much smaller than in the Andalusians, though no figures are obtainable by way of sub-

TABLE VI

SHOWING THE RESULTS OF CERTAIN BLUE, BLUE-SPLASHED AND BLACK ORPINGTON CROSSES AMONG THEMSELVES AND WITH OTHER BREEDS

Group	♂	♀♀		Blue-spl.	Blue	Black	White
1...	Blue Andalusian *(Re)(rE)*	× blue Orpington *(Re)(rE)*	Obtained Theoretical	13 13.75	30 27.50	12 13.75	0 0
2...	Blue-splashed Andalusian *(Re)(Re)*	× black Orpington *(rE)(rE)*	Obtained Theoretical	0 0	37 37	0 0	0 0
3...	Black Andalusian *(rE)(rE)*	× blue-splashed Orpington *(Re)(Re)*	Obtained Theoretica	0 0	14 14	0 0	0 0
4...	White Wyandotte *pp(rE)(rE)*	× blue-splashed Orpington *PP(Re)(Re)*	Obtained Theoretical	0 0	21 21	0 0	0 0
5...	Blue Orpington *(Re)(rE)*	× blue Orpington *(Re)(rE)*	Obtained Theoretical	19 23	58 46	15 23	0 0
6...	Blue F_1 $\frac{\text{wh. Wyand. ♂}}{\text{spl. Orp. ♀}}$ *Pp(Re)(rE)* $X^2 = 4.0505$ P = .2569	× blue F_1 $\frac{\text{wh. Wyand. ♂}}{\text{spl. Orp. ♀}}$ *Pp(Re)(rE)*	Obtained Theoretical	60 50.6250	26 25.3125	24 25.3125	25 33.7500

stantiation. It seemed desirable from several standpoints to ascertain what factors were involved in the production of Orpington blue, and whether the blue Orpington differed from the blue Andalusian in its genetic behavior. A number of matings were accordingly made, the data from which are shown in Table VI.

These data are consistent with the supposition that the factors involved in the production of Orpington blue are identical with those which produce Andalusian blue. The crossing of blue Andalusians and blue Orpingtons gave exactly the same sort of result as that obtained by mating blue Andalusians *inter se,* as shown by group 1. The blue-splashed Orpingtons mated with white Wyandottes gave only blues (group 4) just as did the blue-splashed Andalusians. And finally the F_2 ratio from white Wyandotte $\times$ blue-splashed Orpington crosses gave the same phenotypic classes as were obtained in the F_2 from the white Wyandotte $\times$ blue-splashed Andalusian cross, with a deviation from expectancy as great as would be probable once in four times. It is interesting to note that while the white class is deficient in this case, the blue-splashed class is not.

XII. Data from Blue Leghorn Crosses

In the spring of 1917 there appeared in the large pure-bred single comb white Leghorn flock of the Pabst Stock Farm at Oconomowoc, Wisconsin, two blue females. The flock was not pedigreed and nothing is known of the individual ancestors of these birds. They were of fair Leghorn type and were, as far as known, the offspring of pure-bred white Leghorn parents. Through the courtesy of Mr. Fred Pabst, and Dr. L. J. Cole of the University of Wisconsin, these individuals came into the hands of the writer and were entered on the records of the Department of Poultry Husbandry of the Kansas State Agricultural College as numbers 767 and 768.

Number 767 was a fairly even shade of medium to light

blue when received and showed some evidence of barring, though this was not very distinct. Number 768 was much lighter in shade than 767 and showed no evidence of barring. In contrast with ordinary blue she would, from a little distance, be mistaken for a white. The pigment granules in both cases were round.

The results of mating these birds in various ways are presented in Table VII. The numbers are rather small

TABLE VII

SHOWING THE BREEDING BEHAVIOR OF TWO BLUE LEGHORN FEMALES, WHEN MATED WITH VARIOUS MALES OF KNOWN FACTORIAL COMPOSITION

♂	♀		Blue Splashed	Blue	Black	White
White Leghorn 117M	× 767	Obtained	0	0	0	5
IIPP(rE)(rE)	*iiPP(Re)(rE)*	Theoretical[5]	0	0	0	5
White Leghorn 117M	× 768	Obtained	0	0	0	11
IIPP(rE)(rE)	*iiPP(Re)(rE)*	Theoretical	0	0	0	11
Blue Andalusian 78M	× 767	Obtained	2	4	2	0
PP(Re)(rE)	*PP(Re)(rE)*	Theoretical	2	4	2	0
Blue Andalusian 78M	× 768	Obtained	3	7	4	0
PP(Re)(rE)	*PP(Re)(rE)*	Theoretical	3.5	7	3.5	0
White Plymouth Rock 155M	× 767	Obtained	0	20	24	0
pp(rE)(rE)	*PP(Re)(rE)*	Theoretical	0	22	22	0
White Wyandotte 192M	× 768	Obtained	0	8	8	12
$I^Pi^Ppp(rE)(rE)$	$i^Pi^PPP(Re)(rE)$	Theoretical	0	7	7	14
Blue white Rock ♂ 155M / blue Leghorn ♀ 767	× 767	Obtained	2	6	1	0
		Theoretical	2.25	4.50	2.25	0
Pp(Re)(rE)	*PP(Re)(rE)*					
Black Andalusian 288M	× 768	Obtained	0	25	22	0
PP(rE)(rE)	*PP(Re)(rE)*	Theoretical	0	23.5	23.5	0

but two facts seem fairly evident. First, that 767 and 768 are alike with respect to the factors under discussion in this paper, and second, that they give no indication of being different in their make-up with respect to the factors *R*, *E* and *P* from pure-bred blue Andalusians.

The appearance of the blue offspring of 768 (which it will be recalled was very light) when mated with black or blue Andalusians, was such as to suggest the possibility that accessory factors, necessary for the production of blue of normal shade, were supplied by the Anda-

[5] The theoretical expectancies calculated as for blue Andalusians.

lusian males, though no attempt was made to isolate and identify them.

Since these blue Leghorns arose in an unpedigreed flock, their origin is conjectural. A plausible explanation seems to be that two individuals heterozygous for *I*, the dominant Leghorn factor described by Hadley (1913), which inhibits the production of pigment shown (also by Hadley, 1914) to be normally present in the white Leghorn, happened to mate and that at least one of them carried the factor *R* as a cryptomere. That white Leghorns may sometimes carry the factor seems to be shown by the fact that Dryden (1916, p. 67) secured blue chicks in an F_2 generation from a barred Plymouth Rock $\times$ white Leghorn cross. And further, in the course of the breeding operations reported in this paper, blues appeared in the progeny of a black Andalusian and a white crossbred, the latter being the product of a black Andalusian $\times$ white Leghorn cross. In both cases it appears that the factor *R* must have been brought in by the white Leghorn. This suggestion also involves the assumption that the white Leghorn carries the factor *E*. That this is the case is shown by the fact that in the F_2 from a blue-splashed Andalusian $\times$ white Leghorn cross, the details of which are reserved for later publication, both blacks and blues appeared.

XIII. The Problem of True-breeding Blues

The fact that the blue varieties of both Andalusians and Orpingtons as now constituted do not breed true is a matter of considerable importance to their breeders. It is a heavy handicap to both varieties. While one hundred per cent. of blues may in each case be secured by mating blue-splashed individuals with black, as a matter of practical breeding this mating is seldom made. This is owing to the fact that there are several more or less variable qualities of color for which rigid selection is practised which are not apparent in either the blue-

splashed or blacks. The breeder therefore prefers to use for breeding purposes only those individuals which show the desired phenotypic condition, even though so doing necessitates the discarding of approximately half the offspring. While this leaves a comparatively small number of individuals, as compared with other breeds, upon which to practise selection, the blue Andalusian at least is bred in considerable numbers, thereby indicating its economic desirability and its attractiveness.

As was pointed out in the earlier paper (1918, p. 111) if *R* and *E* are not at identical loci on homologous chromosomes and crossover individuals were found which produce *RE* gametes, the problem of the constant-breeding blue would be solved.

The situation regarding black in rats may not be without its bearing in the present case. Black rats which bred true have been known for some time. Castle (1919) has, however, reported certain races of blacks which failed to breed true. This type of black was tested through several generations by Castle (1919), Ibsen (1920) and Dunn (1920). Blacks mated to blacks quite persistently produced whites, blacks and red-eyed yellows in the ratio of 1 to 2 to 1. Castle (1919) found one possible cross-over individual which died without being tested. Ibsen (1920) has so far failed to find any, and Dunn (1920) reports between one and two per cent. of cross-overs. These cross-overs, which were longer sought for and among larger numbers than has yet been possible with Andalusians, would appear to make it possible to synthesize a true breeding (*i.e.,* homozygous) black, from the line which has not been breeding true through a considerable number of generations.

It is also worth noting in this connection the possible bearing of Sturtevant's (1919) finding families of *Drosophila* carrying at least two definite factors in the second chromosome which almost completely inhibit crossing-over in the region contiguous to their loci.

If, however, after a long-continued search, it becomes increasingly evident that *R* and *E* are indeed allelomorphs, as originally suggested by Bateson and Punnett (1905), it was suggested (p. 113) that hope might be seen in the progressive selection of the darker, that is, more fully pigmented, blue-splashed individuals, there being considerable variation among the latter in this regard.

There is a further possibility which should not be overlooked, namely, that other factors might be found, perhaps in other breeds, which would act on black pigment to give the blue appearance on the one hand, or extend it to give self-colored individuals on the other. If duplicate factors for *E* or *R* should be found, a means of producing the long sought true-breeding blue would seem to be at hand. The fact that three factors are known which produce white in fowls lends emphasis to the possibility. It would seemingly make little difference in the ultimate outcome whether the new factor was linked to *R* and *E*, or was located on a different chromosome pair. In either case it would be possible to get a "self-coloring" and a "bluing" factor in the same gamete which, it appears, has so far not been done.

XIV. SUMMARY

1. It has been shown that the development of black pigment in the blue-splashed, blue and black races of the Andalusian and Orpington breeds, and of black Langshans, depends upon the action of a dominant hereditary factor *P*, for which they are normally homozygous.

2. The allelomorph of *P* is *p*. Individuals homozygous for *p* are white, as in the white Wyandotte and white Plymouth Rock breeds.

3. The extension of black pigment to all feathers of the body, resulting, if no pattern factors are present, in self-colored individuals, depends upon a dominant factor *E*. This factor has been found in the Andalusian, Orpington, white Plymouth Rock, white Wyandotte and black Lang-

shan breeds. Some evidence is presented which indicates its presence in white Leghorns.

4. The blue appearance of blue and blue-splashed Andalusians and Orpingtons, is due to the arrangement and restriction of black pigment, the result of a dominant factor *R*. This factor has also been found in individuals of the white Wyandotte and white Leghorn breeds, though its presence is probably not usual in these breeds.

5. No individuals of the Andalusian, Orpington, white Plymouth Rock, white Wyandotte or black Langshan breeds have been found which did not carry *R*, *E* or both.

6. The mutual relations of *R* and *E* are such that they have never been found together in the same gamete. This indicates that they are allelomorphic, *i.e.*, occupy identical loci on homologous chromosomes, or, each is so closely linked to the recessive allelomorph of the other, (*Re*) and (*rE*), that crossing-over rarely, if ever, occurs.

7. No evidence of crossing-over between *R* and *E* has been found and the tentative conclusion must be in accord with that previously held, that *R* and *E* are allelomorphs.

8. Both *R* and *E* are independent of *P* in their hereditary behavior, though dependent upon its presence for their manifestation.

9. The cooperative influence of the ovary is necessary for a full expression of *R* in the regions of the neck, back and saddle.

10. On the basis of the evidence presented in the body of this paper the genetic formulæ of the breeds and varieties employed, with respect to the factors under observation, are usually as follows: blue-splashed Andalusians and Orpingtons *PP*(*Re*)(*Re*); blue Andalusians and Orpingtons *PP*(*Re*)(*rE*); black Andalusians, Orpingtons and Langshans *PP*(*rE*)(*rE*); and white Plymouth Rocks and Wyandottes *pp*(*rE*)(*rE*).

11. The possibility of the occurrence of factors which duplicate the somatic effects of *R* and *E* is pointed out, and the relation of this possibility to the production of constant-breeding blues briefly discussed.

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INHERITANCE IN NICOTIANA TABACUM. II. ON THE EXISTENCE OF GENETICALLY DISTINCT RED-FLOWERING VARIETIES[1]

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IN our studies of inheritance in *Nicotiana Tabacum*[2] it has been demonstrated that the red flower color of *macrophylla* (U. C. B. G. 22/07) is recessive to the light pink of *angustifolia* (U. C. B. G. 68/07), and the same relations are exhibited by the red of *calycina* (U. C. B. G. 110/05) as contrasted with the light pink of *virginica* (Maryland, U. C. B. G. 78/05). In both cases the F_1 was pink, F_2 conformed to the ratio 3 pink:1 red, and F_3 and subsequent generations yielded data consistent with a single factor difference between these two flower colors. It was also shown that when *macrophylla* was crossed with the white-flowering variety *alba* (U. C. B. G. 30/06), F_1 was pink, F_2 conformed to the ratio 9 pink:3 red:4 white, and F_3 and subsequent generations gave data in agreement with a two-factor difference for this character contrast.

Allard,[3] however, had presented evidence, at first sight contradictory to ours, to the effect that the carmine flower

[1] The experimental data cited herein were obtained from cultures made possible by a portion of the Adams' Fund allotted to the Department of Botany by the Department of Agriculture of the University of California.

[2] Setchell, W. A., T. H. Goodspeed and R. E. Clausen, "A Preliminary Note on the Results of Crossing Certain Varieties of *Nicotiana Tabacum*," *Proc. Nat. Acad. Sci.*, 7: 50–56, 1921. A complete illustrated account of these experiments is in press under the title, "Inheritance in *Nicotiana Tabacum*. I. A Report on the Results of Crossing Certain Varieties," *Univ. Calif. Publ. Botany* 5, no. 17. For descriptions and illustrations of the varieties mentioned in this paper cf. Setchell, W. A., "Studies in *Nicotiana*. I., *Univ. Calif. Publ. Botany*, 5: 1–86, 1912.

[3] Allard, H. A., "Some Studies in Blossom Color Inheritance in Tobacco, with Special Reference to *N. sylvestris* and *N. tabacum*." AMER. NATURALIST, 53: 79–84, 1919.

color of his Giant Red-flowering tobacco is dominant to pink in a simple mono-hybrid relation, F_1 being carmine and F_2 3 carmine : 1 pink. He also crossed this carmine-flowering variety with a white-flowering form and obtained light carmine in F_1 and a distribution which might be taken to conform to the ratio 9 carmine : 3 pink : 4 white. We took these results to indicate that his carmine, which must be very similar to our red, was nevertheless genetically distinct from it. This belief was somewhat strengthened by the fact that our red does not fall upon the carmine of the Ridgway[4] color scale, but lies slightly removed from it between rose red and pomegranate purple, although a difference of this kind might conceivably be due to the effect of differences in the residual genotype. We have, however, a variety, *purpurea* (U. C. B. G. 25/06), which exhibits a red flower color somewhat darker and more intense than that of *macrophylla,* and which some preliminary crosses indicated was dominant to pink and white. We accordingly suggested the following factor formulæ for these four colors:

WWRRPP = carmine
WWRRpp = light pink
WWrrpp = red
wwRRpp = white

In this formulation *WWRRPP,* represents the basic type, carmine in color; *w,* the difference from it which gives white, irrespective of which members of the pairs occupy the *R* or *P* loci; *p,* that which gives pink; and *r,* that which changes pink to red. Obviously white-flowering varieties may be of four different genotypes, viz., *wwRRPP, wwRRpp, wwrrPP,* and *wwrrpp,* but our white variety *alba* was clearly *wwRRpp.* This formulation brings our results into accord with those of Allard and accounts for the existence of genetically distinct red-flowering varieties. We have now obtained further evidence in support of the correctness of this formulation.

[4] Ridgway, R., "Color Standards and Color Nomenclature," 1912.

We found it necessary to use "Cuba" (U. C. B. G. 200/14),[5] another white-flowering variety, in these studies. Since there is the possibility just indicated of the existence of genetically distinct white-flowering varieties, it became necessary to determine the genetic constitution of "Cuba" with respect to the *Rr* and the *Pp* pairs of allelomorphs. A number of crosses were made, therefore, between "Cuba" and *macrophylla* as the starting point for these determinations. In the account which follows H_{174} = *macrophylla* ♀ × "Cuba" ♂ and H_{175} = the reciprocal. In the season of 1919, 50 plants of F_1H_{174} and 100 plants of F_1H_{175} were grown. They were all pink-flowering except that one plant produced a small white-flowering branch in an inflorescence otherwise pink-flowering. This bud variant, one of the few which we have observed in tobacco, will be taken up in a subsequent report. The further data on these reciprocal hybrids are listed in Table I. The F_2 popula-

TABLE I

F_2 AND BACK-CROSS DATA OF THE CUBA-MACROPHYLLA (WHITE × RED) SERIES

Garden Numbers	Parentage	Flower Color			Totals
		Pink	Red	White	
20.075....	$19F_1H_{174}P_{28}W$	59	22	19	100
20.076....	$19F_1H_{174}P_{28}P$	54	22	23	99
Totals for F_2 populations		113	44	42	199
$16F_1H_{182}$..	$15F_1H_{174}$ ♀ × 200/14 ♂	12	—	12	24
$19F_1H_{182}$..	ditto	48	—	50	98
$16F_1H_{183}$..	200/14 ♀ × $15F_1H_{174}$ ♂	12	—	13	25
$19F_1H_{183}$..	ditto	53	—	47	100
$16F_1H_{188}$..	200/14 ♀ × $15F_1H_{175}$ ♂	11	—	13	24
$19F_1H_{188}$..	ditto	50	—	49	99
$16F_1H_{189}$..	$15F_1H_{175}$ ♀ × 200/14 ♂	6	—	19	25
$19F_1H_{189}$..	ditto	53	—	47	100
Totals for back-crosses to white		245	—	250	495
20.059....	$19F_1H_{174}$ ♀ × 22/07 ♂	22	28	—	50

[5] For description cf. Goodspeed, T. H. "Parthenogenesis, Parthenocarpy and Phenospermy in *Nicotiana*," *Univ. Calif. Publ. Botany*, 5: 249–272, 1915.

tions give totals of 113 pink: 44 red: 42 white, whilst the 9:3:4 expectation, disregarding fractions, is 112 pink: 37 red: 50 white. In the back-crosses to both the white and the red parents the data are obviously in satisfactory agreement with the 1:1 expectations. These figures do not establish conclusively the validity of a bigenic formulation for this case, but taken together with the data from the ALBA-MACROPHYLLA series which we have presented elsewhere[6] it seems most reasonable to interpret them in this manner. An alternative mono-hybrid interpretation might be argued, but it would not fit the F_2 totals as well as the dihybrid ratio. The growing of F_3 populations would, of course, soon settle the question, but the results so far secured indicate essential genetical identity of *alba* and "Cuba" in their flower color factors.

In order to demonstrate the difference in behavior of red of *macrophylla* and carmine of *purpurea* we have made parallel crosses between them and a number of other *Tabacum* varieties. The flower colors of these varieties and of their F_1 hybrids with *macrophylla* and *purpurea* are listed in Table II. In each case the F_1 with *macrophylla* was pink but with *purpurea* it was always a full, intense carmine. Among two hundred plants of the CUBA-PURPUREA series one plant appeared which bore

TABLE II

F_1 RESULTS OF PARALLEL CROSSES OF MACROPHYLLA AND PURPUREA WITH A SERIES OF TABACUM VARIETIES

Variety Name and Number	Flower Color	Flower Color of F_1 with Macrophylla	Flower Color of F_1 with Purpurea
angustifolia (U. C. B. G. 68/07).....	Light pink	Pink	Carmine
"Cavala" (U. C. B. G. 72/05).....	Pinkish	Pink	Carmine
"Cuba" (U. C. B. G. 200/14).....	White	Pink	Carmine

carmine flowers on one side and light pink ones on the other. Further studies on this, the most striking case

[6] Setchell, Goodspeed, and Clausen, *loc. cit.*

of somatic variation we have ever observed in *Nicotiana*, are in progress. The F_1 results in themselves sufficiently demonstrate the existence of a genetic difference between the red of *macrophylla* and the carmine of *purpurea*.

We have also secured further data from the CUBA-PURPUREA series which demonstrates the mode of inheritance of carmine when crossed with the same white used in the CUBA-MACROPHYLLA series. These results are set forth in Table III. The totals from the F_2 populations,

TABLE III

F_2 AND BACK-CROSS DATA FOR THE CUBA-PURPUREA (WHITE × CARMINE) SERIES

Garden Numbers	Parentage	Flower Color			Totals
		Carmine	Pink	White	
$19F_2H_{190}$..	$16F_1H_{190}P_8$	58	14	26	98
$19F_2H_{191}$..	$16F_1H_{191}P_6$	28	8	11	47
20.077....	$19F_1H_{191}P_4R$	48	13	39	100
20.078....	$19F_1H_{191}P_4P$	56	13	31	100
Totals for F_2 populations		190	48	107	345
20.060....	200/14 ♀ × $19F_1H_{191}P_4R$ ♂	16	6	28	50
20.061....	200/14 ♀ × $19F_1H_{191}P_4P$ ♂	12	12	25	49
Totals for back-crosses to white		28	18	53	99

190 carmine : 48 pink : 107 white, are to be compared with a 9 : 3 : 4 expectation of 194 carmine : 65 pink : 86 white. The results from the back-crosses, 28 carmine : 18 pink : 53 white, are to be compared with an expectation based on the 1 : 1 : 2 ratio of 25 carmine : 25 pink : 49 white. Pink is again deficient and white in excess, but not to such an extent as to give significance to the figures. Further data from F_3 families would be desirable for completion of the analysis. Thus far the data are in agreement with those presented by Allard for carmine versus pink and white, and they support the conclusion that his carmine variety is identical in its main genetic flower color factors with ours.

The further question now arises as to whether there are any phenotypic differences between carmine and red. There is a detectable difference between the flower color of *macrophylla* and that of *purpurea,* for the former has distinctly more of a purplish tinge and is not quite as intense in coloration as the latter. But these two varieties differ genetically in a large number of other characters. It is not possible, therefore, to decide the question by direct examination, because any distinctions which are found to exist may depend upon differences in the residual genotype rather than upon the specific factor differences which we have studied. Obviously the most satisfactory material for determining the differences between the two colors would be two varieties which had the same residual genotype, but the establishment of such varieties would entail the expenditure of a considerable amount of time and labor. We can, however, obtain some evidence on this problem by comparing the red F_2 segregants of the CUBA-MACROPHYLLA series with the carmine ones from the CUBA-PURPUREA series. In both cases there was a certain amount of variation in intensity of coloration in the F_2 classes, but it was found that, if they were mixed together, it was impossible to separate them again into red and carmine. In cases involving both classes in the same experiment, they would doubtless have to be considered as making up a single phenotype.

We have been interested in determining experimentally whether the morphological similiarities of existing *Tabacum* varieties might safely be taken as an index of phylogenetic affinities. Thus Setchell,[7] commenting on the relationships of *purpurea,* states,

> There are combined in this plant characters of our *N. angustifolia* as to petiole, *N. Tabacum* var. *brasiliensis* as to cucullate tip, tallness, and perhaps also the wing on the petiole, and *N. Tabacum* var. *macrophylla* as to flowers.

It is very natural to regard the sharply constricted leaf-

[7] *Loc. cit.,* p. 11.

base of *purpurea* as a modified petiolate condition, but as a matter of fact our studies have shown that its affinities in this respect lie closer to the sessile leaf type gentically, to which it is recessive, than to the true petiolate class which is dominant to the sessile type. In the present article we show further that the flower colors of *macrophylla* and *purpurea* are distinctly different genetically and their similarity in appearance can not be regarded as an indication of phylogenetic relationship. It is, therefore, evident that any taxonomic system which proposes to portray the phylogenetic affinities of the polymorphic assemblage of *Tabacum* varieties must be derived from genetic studies of character differences.

Allard has suggested the use of these flower color forms for instructional work in genetics. The demonstration of these additional relations increases their interest and value for such purposes. Among other points of interest a cross between *macrophylla* and *purpurea* should give a carmine F_1 and the rather unusual F_2 segregation ratio of 13 carmine (and red) : 3 pink. We have verified the production of carmine F_1 in this cross, but have not yet grown the F_2 progeny. The ease of hybridization, the readiness with which large quantities of guarded seed may be secured, and the extremely long period over which the seed of tobacco retains its viability may be urged as additional advantages in its utilization. Where greenhouse and garden space is available for their growth—plants may easily be grown to maturity in six-inch pots—these varieties and their hybrids would provide excellent material for practise in hybridization and for demonstrations of segregation and unique character interrelations. While there is a certain amount of variation within the several phenotypes here considered, viz., carmine, red, pink, and white, it has not been found to interfere seriously with segregation into the main color classes.

AN ANALYSIS OF THE RELATION BETWEEN GROWTH AND NUCLEAR DIVISION IN A PARASITIC INFUSORION, OPALINA SP.[1]

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THIS investigation was undertaken for the purpose of analyzing the relation between growth and nuclear division in a species of *Opalina* of the frog during the growth period in the tadpole. The multinucleate condition of *Opalina* and the absence of cell walls render it of particular value as material for the study of the phenomena involved in nuclear division and growth. The specimens used in our investigations were obtained by Dr. Charles E. Simon from tadpoles collected at Chester, Nova Scotia, during the summer of 1920. Unfortunately we are unable to state either the species name of the *Opalina* or that of the host. Dr. Maynard M. Metcalf, who has examined the slides, thinks the *Opalina* is probably an undescribed species. The material was well fixed in Schaudinn's solution and beautifully stained with iron-hematoxylin.

A sufficiently large number of specimens (455) were drawn with a camera lucida so as to furnish reliable results when measurements were treated by statistical methods. The area of the drawings was determined with a planimeter and the correlation with the nuclear number determined. Table I is the correlation table for the nuclear number and area of 341 specimens. The area of the drawings, which were made at a magnification of 650

[1] From the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

diameters, is given in square millimeters. The coefficient of correlation is remarkably high, namely, $.755 \pm .016$; this proves that an increase in size is accompanied by a corresponding increase in nuclear number. The rest of the specimens that were measured, 114 in number, were drawn at a magnification of 1400 diameters. The coefficient of correlation of this lot was found to be $.875 \pm .015$.

TABLE I

CORRELATION TABLE FOR NUMBER OF NUCLEI AND AREA OF 341 SPECIMENS

The area is given in sq. mm. and obtained from camera-lucida drawings made at a magnification of 650 diameters. Coefficient of correlation $.755 \pm .016$.

Area	Number of Nuclei																	
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	27	28	29	
300+		1																1
400+	1	1	2				1											5
500+		1	1	1	1													4
600+	1	7	7	3	1													19
700+		4	6	12	2													24
800+		2	12	13	5	3		1										36
900+			6	25	8	6	1											46
1000+			1	13	14	9	8											45
1100+		2		3	12	20	6	4	1									48
1200+			1	1	8	3	5	5	1									24
1300+	1	1	1	1	1	5	4	4	1		2							21
1400+				1	1	3	4	5	4	2								20
1500+				1				4	1	1	1	3						11
1600+							1	1	3	2	1		1					9
1700+			1		1		1	1							1			4
1800+			1						2	2					1			6
1900+			1	1		1	1		1	1	1							7
2000+													1					1
2100+									1	1		2						4
2200+									1									1
2400+								1					1	1				3
2800+																	1	1
3200+																1		1
	3	19	40	75	54	50	32	26	16	9	5	5	3	1	1	1	1	341

Metcalf[2] has pointed out that in multinucleate Opalinas the nuclei within a single specimen may be in different stages of division at one time. This we have found to be true also of the nuclei during the growth stages in the tadpole—a condition that has enabled us to analyze with

[2] Metcalf, M. M., 1909, "*Opalina*," *Arch. f. Protist.*, 13: 195–375. Especially p. 269.

considerable accuracy the exact relation between cytoplasmic mass and nuclear division. For example, among the specimens with four nuclei, there were a few with three "resting" nuclei and one nucleus in division (Fig. 3); obviously one of the four nuclei is undergoing division before its three sisters. If the sum of the areas of a number of specimens in which there are four nuclei of equal size (Fig. 2) is divided by the total number of nuclei, a fairly accurate idea may be obtained of the amount of cytoplasm associated with each nucleus. According to the nucleo-cytoplasmic relation theory[3] an increase in the amount of cytoplasm as compared with the amount of nuclear material furnishes the stimulus which initiates nuclear division. A comparison between specimens with four equal nuclei, and specimens with four nuclei one of which is undergoing division, should reveal approximately the increase of cytoplasmic substance necessary to inaugurate nuclear division. A number of cases of this sort were available in our material and were studied with the following results.

Table II shows the relations between area, and number, volume and surface of the nuclei in the 207 specimens that could be used for this purpose. The measurements were made of camera-lucida drawings at a magnification of 650 diameters. Beginning with the group of 15 at the top of the table we can make the following comparisons.

1. Fifteen specimens, each with 4 equal nuclei (Fig. 2), have an average area per nucleus of 176.1 sq. mm.; 10 specimens, each with 3 equal nuclei and a fourth nucleus in division (Fig. 3), have an average area per nucleus of 185.0 sq. mm. The specimens in which division has been initiated have an average area per nucleus 8.9 sq. mm. greater than those with an equal number of nuclei, none of which are in division. We have used the area through-

[3] For a recent discussion of this theory see Hegner, R. W. 1920, "Relations between Nuclear Number, Chromatin Mass, Cytoplasmic Mass, and Shell Characteristics in Four Species of the Genus *Arcella*," *Jour. Exp. Zool.*, 30: 1–95.

TABLE II

TABLE SHOWING THE RELATION BETWEEN AREA AND NUMBER, VOLUME AND SURFACE OF THE NUCLEI

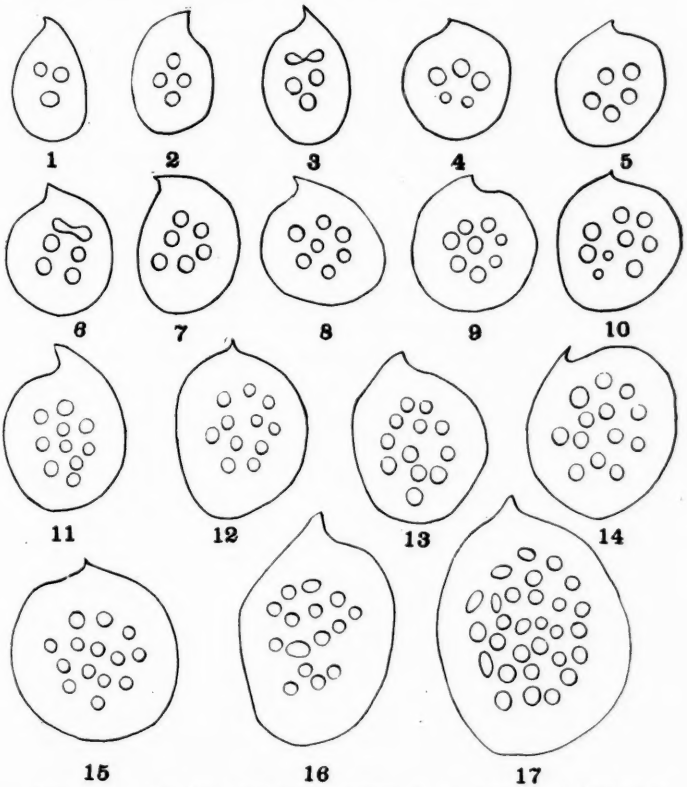
The area, volume and surface of the nuclei were computed from camera drawings made at a magnification of 650 diameters.

Number of Specimens	Number of Nuclei	Average Area in Sq. Mm. at Magnification of 650 Diameters	Average Area per Nucleus in Sq. Mm. at Magnification of 650 Diameters	Average Volume of Nuclei in Cubic Mm. at Magnification of 650 Diameters	Average Volume per Nucleus in Cubic Mm. at Magnification of 650 Diameters	Average Surface of Nuclei in Sq. Mm. at Magnification of 650 Diameters	Average Surface per Nucleus in Sq. Mm. at Magnification of 650 Diameters
15	4 (equal in size)	704.2	176.1	215.49	53.87	261.32	65.33
10...	4 (one in division)	739.9	185.0				
13...	5 (two small)	851.5	170.3	257.11	51.42	321.10	64.22
16...	5 (equal in size)	860.5	172.1	281.91	56.38	345.01	69.00
17...	6 (two small)	951.4	158.6	273.69	45.62	363.85	60.64
49...	6 (equal in size)	926.7	154.5	264.83	44.14	352.89	58.81
5...	6 (one in division)	1,032.5	172.1				
11...	7 (two small)	993.5	141.9				
31...	7 (equal in size)	1,066.7	152.4	269.17	38.45	379.68	54.24
10...	7 (one in division)	1,176.7	168.1				
30...	8 equal	1,127.9	141.0	336.53	42.07	458.93	57.37
207							

out our work as a measure of cytoplasmic mass, hence it appears from the results of our measurements that an increase in mass per nucleus represented by an increase in area within the limits of 8.9 sq. mm. is the stimulus that initiates nuclear division. The exact mass of cytoplasm represented by this increase in area of 8.9 sq. mm. might easily be obtained under more favorable circumstances.

2. When we compare the measurements of the 10 specimens with 4 nuclei, one of which is dividing (Fig. 3) with 13 specimens of the stage immediately following, with 3 large nuclei and two that have just reorganized after division (Fig. 4), we find that although the latter average 111.6 sq. mm. larger per specimen the average area per nucleus is 14.7 sq. mm. less. Thus there has been an

actual increase in size but a decrease in the mass of cytoplasm associated with each nucleus.



FIGS. 1–17. Outline drawings of stages in the growth of *Opalina* sp. made with a camera lucida at a magnification of 650 diameters and reduced to a magnification of 325 diameters.

3. During the growth of the two small nuclei to their full size (Figs. 4–5) the size of the organism increases from an average area of 851.5 sq. mm. per specimen to an average area of 860.5 sq. mm., or an average area per nucleus of from 170.3 sq. mm. to 172.1 sq. mm. Although an increase in size has taken place, the average area per nucleus of 172.1 sq. mm. in specimens containing 5 full-

sized nuclei is less than that of specimens with 4 full-sized nuclei, *i.e.*, 176.1 sq. mm.

4. A further increase in the average size of the specimens occurs between the stage with 5 nuclei of equal size (Fig. 5) and that with 6 nuclei, two of which have just emerged from mitosis. Measurements give for the former an average area of 860.5 sq. mm. and for the latter 951.4 sq. mm. The specimens with the two small nuclei, however, possess as before (see (2)) a lower average area per nucleus, *i.e.*, 158.6 sq. mm. as compared with 172.1 sq. mm. in specimens with 5 full-grown nuclei.

5. The measurements of the next stage, *i.e.*, specimens with 6 nuclei of equal size (Fig. 7), are more difficult to explain, since the average area of the specimens (926.7 sq. mm.) is actually less than that of the younger specimens (see (4)) with 4 large and 2 small nuclei, and the average area per nucleus falls from 158.6 sq. mm. to 154.5 sq. mm. These results may be due to a thickening of the entire animal which would increase the mass and tend toward a decrease in area or the nucleo-cytoplasmic relation may change as the animals become older. That there is an actual decrease in the average area per nucleus as growth proceeds is indicated by the measurements of later stages as given in Table III. This table shows a decrease per nuclear area from 186 sq. mm. in specimens with 4 nuclei to 96.8 sq. mm. in specimens with 29 nuclei. That this decrease is gradual is indicated when averages are made of three successive groups containing each a larger number of specimens. Thus the 4, 5, and 6 nucleated groups containing 134 specimens have an average area per nucleus of 173.8 sq. mm., the 7, 8, and 9 nucleated groups containing 136 specimens have an average area per nucleus of 144.4 sq. mm., the 10, 11, and 12 nucleated groups containing 51 specimens have an average area per nucleus of 143.5 sq. mm., and the 13, 14, and 15 nucleated groups containing 13 specimens have an average area per nucleus of 128.3 sq. mm.

TABLE III

TABLE GIVING THE AVERAGE AREA PER SPECIMEN AND PER NUCLEUS OF 338 SPECIMENS DRAWN WITH A CAMERA LUCIDA AT A MAGNIFICATION OF 650 DIAMETERS

Number of Specimens.	Number of Nuclei.	Average Area per Specimen in Sq. Mm. at Magnification of 650 Diameters.	Average Area per Nucleus in Sq. Mm. at Magnification of 650 Diameters.
19.....	4	744	186.0
40.....	5	891	178.2
75.....	6	944	157.3
54.....	7	1,069	152.7
50.....	8	1,150	143.7
32.....	9	1,231	136.8
26.....	10	1,395	139.5
16.....	11	1,635	148.6
9.....	12	1,709	142.4
5.....	13	1,572	120.9
5.....	14	1,801	128.6
3.....	15	2,032	135.4
1.....	16	2,452	153.2
1.....	17	1,806	106.2
1.....	28	3,277	117.0
1.....	29	2,806	96.8

6. By the time one of the nuclei of the six-nucleated stage has been stimulated to division the average area per nucleus increases again to 172.1 sq. mm. After this division is completed and seven nuclei are present, two of them small, the average area per nucleus, as was to be expected, decreased to 141.9 sq. mm. During the period necessary for the two small nuclei to reach their full size (Fig. 8) the area increases again to 152.4 sq. mm. A further increase to 168.1 sq. mm. occurs by the time sufficient growth takes place to stimulate one of these seven nuclei to divide, and a decrease (to 141.0 sq. mm.) again takes place when this stage evolves into that with eight nuclei (Fig. 9).

The two curves in Fig. 18 show clearly the increase in area per nucleus up to the point where one nucleus divides, then a conspicuous decrease following nuclear division, and subsequently an increase during the period when the nuclei resulting from division regain their full size, ending in a size at which the area per nucleus is approximately that present at the beginning.

Figure 19 illustrates the fact that the size of the entire specimens increases during nuclear multiplication and growth, but that the area per nucleus remains almost constant.

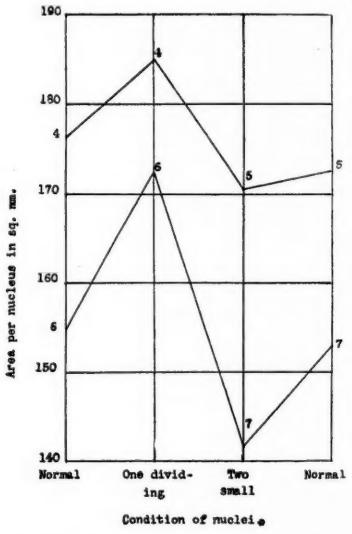


FIG. 18. Curves showing the changes in area per nucleus that accompany changes in nuclear number and condition. The numbers 4, 5, 6, and 7 indicate the number of nuclei present.

7. The series of measurements of these specimens affords an explanation of the reason why nuclear division in *Opalina* is not synchronous. According to the nucleocytoplasmic relation theory, as soon as the mass of cytoplasm has increased to a certain point nuclear division is initiated. The necessary increase to furnish this stimulus in *Opalina* may be determined approximately from our data by comparing measurements of specimens in which the nuclei are all equal in size with those in which nuclear division has been inaugurated. Such a comparison gives the following results.

Nuclei	Average Area per Nucleus	Difference in Area — Amount Necessary to Stimulate Division
4 (equal)	176.1 sq. mm.	
4 (one in division)	185.0 sq. mm.	8.9 sq. mm.
6 (equal)	154.5 sq. mm.	
6 (one in division)	172.1 sq. mm.	17.6 sq. mm.
7 (equal)	152.4 sq. mm.	
7 (one in division)	168.1 sq. mm.	15.7 sq. mm.

These figures, of course, indicate only the relative increase necessary to stimulate nuclear division; the actual increase could be determined by measuring accurately the mass of cytoplasm in each case.

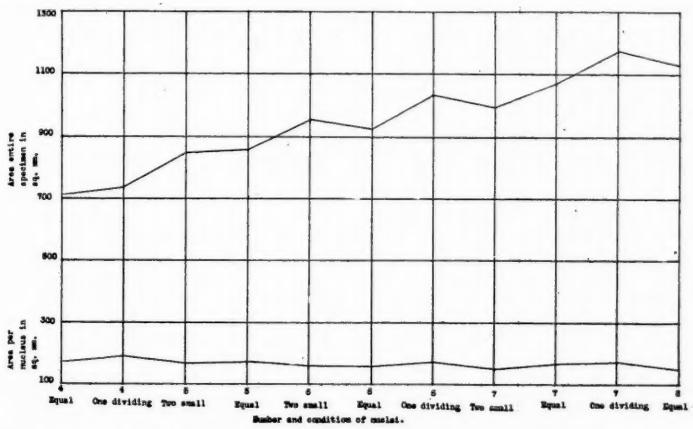


FIG. 19. Curves showing the increase in the area of specimens with increase in age and the constancy of the area per nucleus.

In looking over our camera drawings it was noticed that usually only one nucleus was in division in any one specimen (Figs. 3 and 6) and that in many cases two of the nuclei in a specimen were smaller than the rest (Fig. 4), indicating that they were daughter nuclei that had just emerged from mitosis. Of a total of 137 specimens in which nuclei were found in division, 109 contained one division figure, 19 contained 2, 8 contained 3, and 1 contained 4. Furthermore, those containing more than

one division figure were usually older than those containing one only. Thus the average nuclear number of specimens with one dividing nucleus was 7.2, with two dividing nuclei, 8.1, with three dividing nuclei, 9.3, and with 4 dividing nuclei, 10. These data favor the conclusion that the stimulus that initiates nuclear division acts as a rule on only one nucleus at a time and that the division of this nucleus restores the nucleo-cytoplasmic ratio. When this ratio is again disturbed by an increase of the cytoplasmic mass another nucleus is stimulated to divide. Division of two or more nuclei synchronously may be due to the more rapid growth, the larger specimens in which this usually occurs, or to the greater chances of two or more nuclei reacting to the division-stimulus when a large number of nuclei are present in a single specimen. There is some evidence that the nucleus that undergoes division is the one with the greatest amount of cytoplasm surrounding it, but this could not be determined definitely. No regular distribution of the nuclei was evident. It is interesting to note in this connection that during the embryonic development of many animals nuclear division occurs in all cells at nearly the same time. This is especially interesting in the case of certain insects, in the eggs of which nuclear division proceeds synchronously without the intervention of cell walls until thousands of nuclei are present in a single egg.[4] An increase of cytoplasm over nucleus may also, in these insect eggs, stimulate nuclear division, since after each division the mass of cytoplasm surrounding each nucleus is increased by the addition of new material elaborated from the yolk substance in which it is situated.

8. The average total volume of the nuclei of certain specimens, average volume per nucleus, average total area of the surface of the nuclei and average area of the surface per nucleus were measured in cubic millimeters and square millimeters from our camera drawings which

[4] Hegner, R. W., 1914, "Studies on Germ Cells," *Jour. Morph.*, 25: 375–509. Especially pp. 408–413.

were magnified 650 diameters. These data are shown in Table II. They indicate one point of considerable interest and that is the fact that as the number of nuclei increases their average volume and surface decrease.

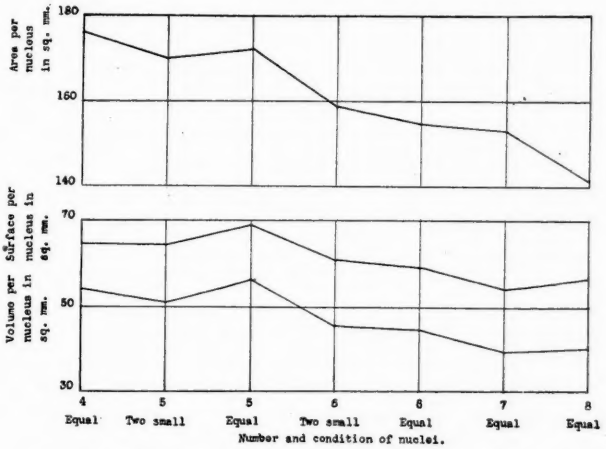


FIG. 20. These three curves show that decreases in the volume and surface of the nuclei are accompanied by decreases in area per nucleus.

Thus in specimens with 4 nuclei of equal size each nucleus has an average volume of 53.87 cu. mm., in specimens with 6 nuclei the average volume per nucleus decreases to 44.14 cu. mm., and in specimens with 8 nuclei the average volume per nucleus decreases still further to 42.07 cu. mm. Similar results were obtained from measurements of the surface of the nuclei, but the decrease is not so great since the volume decreases as the cube whereas the surface decreases only as the square. As the table (II) shows the average surface per nucleus in sq. mm. decreased from 65.33 sq. mm. in specimens with 4 nuclei, to 58.81 sq. mm. in specimens with 6 nuclei, and 57.37 sq. mm. in specimens with 8 nuclei. This decrease in volume and surface may account for the fact noted previously (in (5)), that the area per nucleus decreases in specimens with nuclei all equal in size as the number of

nuclei becomes greater with advancing age. Since the volume of the nuclei is less in these older specimens the amount of cytoplasm associated in normal nucleocytoplasmic relations with them is less and the area of the specimens per nucleus decreases accordingly.

The curves in Fig. 20 bring out clearly the relation between area and volume and surface of the nuclei during the growth period. The average area per nucleus decreases as the number of nuclei increases, but at the same time there is a corresponding decrease in both volume and surface of the nuclei, thus maintaining approximately the initial relation between nucleus and cytoplasm.

SUMMARY

(*a*) A high correlation exists between nuclear number and cytoplasmic mass (as indicated by area) during the growth of *Opalina* sp. The coefficient of correlation in one lot of 341 specimens was $.755 \pm .016$ and in another lot of 144 specimens was $.874 \pm .015$.

(*b*) By comparing the area of various stages with the number, size, state of division, volume and surface of the nuclei the following conclusions were reached. (1) Nuclear division is stimulated by an increase of cytoplasm that may be determined approximately. (2) As the organisms increase in age the nuclei decrease in volume and surface; this is accompanied by a corresponding decrease in the area per nucleus, indicating that the nucleocytoplasmic relation is maintained. (3) Nuclear division is not synchronous because one nucleus is usually stimulated to divide before the others, and this division is sufficient for the time to reestablish the normal relation between nuclei and cytoplasm.

AMERICAN FOLLICULINAS: TAXONOMIC NOTES

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THE ciliated infusorian *Folliculina*, "the bottle animalcule," was first recorded by O. F. Müller in 1781 and was by him described amongst 75 Vorticellas as one living in an ampulla or bottle. The name *Folliculina* was suggested by Lamarck in 1816, yet he, having no personal observations of the creature, placed it among the rotifers. However, the true affinities of the bottle makers with the stentors became evident to Claparede in 1858, though members of the group have been referred by others to such genera as *Cothurnia, Ascobius* and *Vaginicola* from insufficient knowledge of the animal within the bottle. Two observers emphasized the nature of the bottle *maker* rather than the bottle itself in seeking to establish for it the generic names Freia and Lagotia; the former given it by Claparede as having the "forme gracieuse et elegante d'une Freia," and the latter by Wright, in the same year, from the long lobes of the animal that resemble the ears of a hare.

The only careful studies of the animal have been made by Stein in 1867 and by Möbius in 1887. The latter was inclined to regard all the then known species as local varieties of the original *Vorticella ampulla* O. F. Müller. But recently Carl Dons in Norway has made very minute study of the bottles as found in many localities and has come to the conclusion that these alone may be used as sufficient basis for establishing species, even without the animal, which, to be sure, is rarely preserved in museum material. He would recognize some ten species, most of which he finds in Norway, but many of which are widespread over the world.

These ten species he proposes to distribute amongst four new genera as follows: The original forms of Müller

with simple bottles of very wide diameter and short plain neck with no lips retain the name *Folliculina*. The type species *F. ampulla* being found in Norway, Denmark, at Kiel and in the Adriatic, while a peculiar species *F. paguri* from the west French coast was described by Giard in 1880 as *Pebrilla paguri*.

Forms with more elongated bottles and with longer necks, often marked with a spiral ridge, as well as provided with a collar or lip to the mouth of the bottle, he calls *Semifolliculina*. The best described is his *S. gigantea* from Norway, as well as the South Polar Sea, while *S. boecki* is the name chosen for an old and widely distributed form occurring in Norway, Denmark, the Adriatic, North America, Formosa, as well as both North and South Polar Seas. *S. similis* is an aberrant form from the South Seas and *S. spirorbis* is the smallest form, with very narrow neck, found both in Norway and upon material from North America, East coast.

Long, straight bottles with the bottom thickened as a falsification of the actual content as judged from the outside are placed in the new genus *Pseudofolliculina*, represented by the large *P. mellita* from the South Polar Seas and by the similar species *P. arctica* from the north of Norway. The remaining bottle animals have been described as having some sort of a valve or set of membranes in the neck of the bottle, and these Dons includes in the genus *Parafolliculina*. *P. amphora* he has described minutely from Norway and from Iceland, and *P. violacea* is a well-known form from west France, the Adriatic, west Australia, as well as Norway.

All these bottles are minute, less than a millimeter long, and though made of a chitin-like material that admits of long preservation in museum jars, they have generally been overlooked, though so common in many parts of the world from the surface down to considerable depths attached to solid objects, such as shells, stones and plants, either singly or in large aggregates or settlements. Moreover, certain species have been described

in England, France and Switzerland as occurring in entirely fresh waters. The above review of the known species and their distribution does not do justice to Strethill Wright's careful description of the animals that make the cases or bottles on the British coasts where he found species not entirely synonymous with the above-cited ten.

The first record of the occurrence of any of these bottle animalcules along the American coasts seems to have been that of Leidy, who, in July, 1859, at Newport, Rhode Island, found attached to *Anomia-serpula* on dead clam shells dredged by Mr. Powel " a singular and beautiful animal " in a vase-like tube and with the same general structural appearance as that of the stentors.

He recognized its resemblance to *Chætospira mueleri* Lachman and its alliance with the stentors and suggested the name *Freyia Americana* for it. Later, according to Ryder, Leidy considered his species to be the same as the European *Folliculina ampulla.* It was not till 1880 that the bottle animalcule was again observed in American waters, and then Ryder on the western shore of the Chesapeake Bay, probably at St. Jerome, St. Mary's County, found a different form of bottle and animal which he identified with the *Freia producta* of Strethill Wright.

The occurrence of this form of bottle animalcule in other parts of the estuaries of the Chesapeake was pointed out in 1914, 1915 by Andrews under the name *Folliculina,* but without determination of the species described. Meantime it was known to workers at Woods Hole, Mass., that *Folliculina* occurred there also, though no published accounts appeared. Dons has recently mentioned the observation of a *Folliculina* upon material from the east coast of America and refers another species to this coast, probably from the above account of Leidy.

We know merely that the animal has been found at Woods Hole and Newport and from waters of the Chesapeake. Considering that the animal is so very widely distributed in Arctic, Antarctic and northeast Atlantic

regions, as well as the Adriatic, it is probably common along many American coasts where as yet overlooked.

In seeking to refer the different forms of the bottle animalcule found thus far in American waters to known or new species we are confronted with ignorance of the anatomy and the life history of the animals and thrown back chiefly upon the secreted bottle or case, since it is this alone that is commonly preserved, and since this also presents preserved characters of form and proportions.

In the life history as known there has been no restriction of the possibilities of form and size change possible to a single individual. We know that there are free-swimming forms as seen by Claparède and more fully studied by Wright and confirmed by Andrews and by Penard.

In some cases these swarmers arise from fission of the parent as Möbius found to be true, but in many instances the free-swimmers that swarm out are only the old forms transformed into simpler larval shapes that have later to make new bottles and then become again complex in structure. In the former case the result of fission is one free-swimmer of small size and one remnant individual left to complete its perfect organization in the old bottle. There are thus large and half-sized forms: both perfect sedentary individuals and imperfect swimming larvæ. Moreover, we find that not only may each individual greatly change its shape from muscular contraction, but may change both shape and bulk under conditions other than the optimum of good feeding environments. Nothing is known of any conjugation and any influence this may have upon form and size.

While it is easy to assume that all the known forms of bottles may prove to be the products of but one and the same species widespread all through the various oceans of the world, evidence for this is lacking, and not having sufficient anatomical basis for classification, we must as a practical expedient adopt the plan of Carl Dons and determine the species by the form and size of the bot-

tles—ceding the point that these species may well have but a very temporary and artificial value.

That the species determined from characters of the temporary dwellings of the animal may, however, prove to be real species is indicated by the following considerations.

The bottle or case is a secretion from the surface of the animal, and, as seen by Wright in 1861, the bottom part, or sac, as we call it, is made first, and then the neck or tube added, and finally the lip at the mouth of the bottle. In making the sac the animal flattens its body and assumes the size and the form that the sac will have when it is poured out and hardened all round about the body in this shape, leaving only the blunt anterior end of the body free from secretion, so that the hardened sac comes to have a hole in the anterior end and is a bilaterally symmetrical product duplicating the form and proportions of the animal as if the latter had been cast in the sac as in a mold. The form of the sac is the form of the animal at that period of its life cycle. Subsequently the size, length, spiral ornamentation, if any, and the perfection of the funnel or lip of the tube are all representations of the habit of the animal in the consecutive phases of the manufacture of the tube. The angle that the neck of the bottle, or tube, makes with the body of the bottle, or sac, is fixed by the degree to which the animal contracts its anterior part to rear it up away from the surface of attachment and general plane of the sac; the diameter of the tube is that of the head end of the animal; its circular section is that of the head end; its length is that of the gradual elongation of the entire animal which carries the head end gradually ever farther away from the foot end till the maximum length is attained; the spiral character of the tube, when present, is determined by the rotation of the head end and by the localized and radially differentiated selective secretions and contractions of parts of the head end; the final lip of the tube is added by special change of shape of the head end which

assumes a mushroom form and secretes from its under surface. At any one period of making of tube the tube expresses the resultant of the two components, contraction and secretion by the body at that moment.

While the entire animal never has the shape of the tube, yet each part of the tube exactly fits the head end of the animal as it progresses away from the foot in coordination with the elongation of the whole animal. The completed structure represents a solidification of the form rhythms of the animal as does the shell of a gasteropod or the successive exoskeletons of a lobster or the hard envelope of a rhizopod, and differs from most of these chiefly in that the animal only temporarily assumes the forms expressed by the dwelling, and later lives freely movable in the dwelling and capable of leaving it by simply detaching the foot-end from the bottom of the sac.

How precisely the bottle represents the animal was seen in one instance when camera drawings of two successive bottles made by the same animal exactly coincided. If, then, the different shapes and sizes of bottles do not mean different species, it is because the different activities and forms of these animals are not specific, but only varietal or individual differences, or differences due to changing conditions, such as food, or to different successive internal states connected with internal rhythms. In ignorance of the possible changes of form any animal may go through, we may, for practical purposes, follow Carl Dons in describing the bottles as expressions of forms that *may* be specific in value.

Of the anatomical characters in *Folliculina* that may be made use of in classification, the nucleus has been emphasized by Dons, who would regard a moniliform nucleus as the attribute of one group of folliculinas as represented by Mueller's original species, while all others have a single lobed simple nucleus. But this is of no avail, for in the first place, the folliculina thoroughly studied by Möbius had moniliform nucleus but its dwelling can not at all be confounded with that of Mueller's

Folliculina, and Ryder expressly mentions the long-beaded nucleus in the form *Freia producta,* which is most remote from Mueller's simple form; and, in the second place, I find that the commoner Chesapeake *Folliculina* has a moniliform nucleus and is by no means close to the *Folliculina* of Mueller.

Thus the moniliform nucleus is not restricted to animals in sacs of the Mueller type. Moreover, observation shows me that in the commoner Chesapeake *Folliculina* the nucleus may pass from the moniliform shape to more and more simple shapes, resembling the elliptical nucleus of so many other species; that is, just as was to be expected from the findings of Johnson in *Stentor,* the form of the nucleus is not constant, but a very long moniliform nucleus may fuse into a short elliptical shape. The same change was observed by Sahrlage.

Whether the nucleus is condensed or nodulated can then be but a poor basis for classification of the Folliculinas.

Till recently no micronuclei have been described in Folliculinas, though known in *Stentor,* and Carl Dons has used this as basis for separating the Folliculinas from the *Stentor* family; however, in two forms of Chesapeake Folliculinas I find minute darkly staining bodies associated with the macronucleus which may well be micronuclei, though their function has not been observed.

The only other anatomical character available seems to be the form of the anterior part of the body which is in some Folliculinas a funnel, and in others a funnel with two sides, more produced so that they may even form long arms likened by Ryder to obstetrical forceps, and again, in another species, by Wright, to the long ears of a hare.

But here again I find that an animal may have in its periods of maximum expansion and feeding activities exceedingly long arms, which in retracted states during adverse conditions may be very greatly reduced and modified in form and proportions, and there are also transi-

tion stages constantly found in which the arms are either being regenerated, or "rediffferentiated," or reduced, to vanishing point.

It is precisely the great development of the edges of the funnel that makes the folliculina an advance upon the simpler state found in the stentors, so that the more simple folliculinas are those with a peristome readily referable to the stentor state, while the very highly differentiated folliculinas with extremely long ligulate lobes right and left from the edge of the funnel are the most remote from the stentors.

While in classification the relative amount of development of these lobes is evidently of great importance, it will require observation of many living specimens, as a rule, to determine whether a given specimen has short lobes from its present stage of development in the individual life cycle or from its permanent place in the stage of evolution from the stentor-like ancestor. On the other hand, the presence of long ligulate lobes will at once determine a high stage of individual and phyletic advance and place the specimen in the highest group of anatomical development.

But until the possibilities of change of form in each individual are known, and in the probable possession of only poorly preserved specimens, the practical expedient will be to adopt much of the procedure of Dons in making use of the forms of the dwellings in the description of what may for the present be regarded as species within the group of Folliculinas.

Relying, then, largely upon the bottles as indicative of specific differences, in the American Folliculinas, so far known, we may tentatively adopt the general subdivisions of Carl Dons, retaining the genus *Folliculina* for the small, very wide sacs with short simple tubes.

No form of this restricted genus has thus far been reported from the American coasts. Whether such forms are anything more than starved, depauperate or imperfectly developed Folliculinas may well be doubted.

The genus *Semifolliculina* he has invented for bottles of narrower form with longer necks, often spirally ornamented and provided with a collar or lip. He says that one of the most widely distributed, *Semifolliculina boecki*, occurs in North America, and probably he had in mind the specimens described by Leidy as *Freia americana* as above related. However, the description given by Leidy speaks of the "convolvulus-like mouth" of the tube, which is plainly shown in an unpublished sketch (Fig. 414, Vol. V, of Leidy's MS. drawings) made by Leidy, which, with his notes, were kindly copied for me by Professor J. Percy Moore, who succeeded in finding it among unpublished material left by Leidy. This sketch also shows not only transverse lines on the tube, but longitudinal lines that may be compared to those of Dons's *Semifolliculina gigantea* (though Leidy may have drawn some of the lines to bring out curvature). At all events the very wide lip of the tube recalls the lip of *S. gigantea*. The size of the animal stated in Leidy's published notice is almost a fifth of a line. In his manuscript certain measurements would on this basis mean that: length of animal is 416 μ expanded, but 298 contracted; length of entire bottle 416 μ; width of animal 166 μ, but at narrow neck below terminal funnel 83; width of expanded funnel and lobes, that separate "like a labiate flower," is 139 μ. From the sketch the evident nucleus is a rounded mass that might be 40 μ in diameter.

Dons has given measurements of various parts of bottles of *Semifolliculina boecki* in contrast with those of *S. gigantea* from which it appears that the animal seen by Leidy was rather larger than the tubes of *S. boecki;* thus the combined lengths of sac and tube in *S. boecki* are 265–410 and for *S. gigantea* are 250–1000; the width of sac, 105–135 in the former and 230–300 in the latter. Leidy's animal was apparently 166 wide and its bottle 416 long. Thus both width of lips of tube and dimensions as far as known tend to place Leidy's animal in *S. gigantea* rather than in *S. boecki*. However, the fig-

ure and the description of " vase-like tube " emphasizes the short neck of the bottle and makes the reference to *S. gigantea* doubtful.

A second *Semifolliculina* is known on the statement of Dons to have been found in material from the eastern coast of America, and this is his *S. planorbis,* characterized by very narrow tube and wide sac. This is suggestive of some of the apparently depauperate or dwarf forms occasionally met with in the Chesapeake and may prove to be but a transitory condition due to conditions of food or other factors and not a permanent form.

The folliculina found at Woods Hole, Mass., has not been described. In 1914 Dr. Elmer J. Lund observed these Folliculinas on stems of *Campanularia, Eudendrium* and *Bugula* from the wharf of the Bureau of Fisheries and in letters to me sketched the sac and tube with expanded lips and absence of spiral. Apparently the form and proportions are much as in the sketch of Leidy, but with longer tube. Professor Lund observed the free-swimming forms several times during the summer in various cultures and saw the formation of new bottles in June.

Some preparations of *Obelia* mounted many years since, probably at Woods Hole, Mass., about 1888, and very likely by Professor Brooks, have yielded me several specimens of *Folliculina* that are evidently the same forms as those seen by Lund and probably the same as those of Leidy. The animals are exceptionally well preserved, in dwellings that give the following measurements:

1. One sac attached its whole length to *Obelia* is 175 μ long and 50 μ deep, while the tube arising from it nearly at right angles is only 50 μ above the top of the sac, is 37 μ wide and flares out at the lip to a width of 62 μ. The animal drawn into its sac is 162 μ long and 35 mm. in greatest diameter, with spheroidal macronucleus 15 μ in diameter. The ligulate lobes are 75 μ long and 12 wide and plainly show the characteristic adoral zone proceed-

ing from the vestibular spiral out to the tip of the left arm, thence back to the dorsal curve, out to tip of right arm, and then along some distance dorsal to the ventral edge of the right arm, to end abruptly at entrance to funnel.

2. Another specimen seen from above has 250 μ length of tube and sac combined (100 tube, 150 sac), width of sac 75 μ, width of tube 40 μ, width of flared lips 75 μ. The macronucleus is 20 by 15 μ and the oral lobes the same size as in preceding specimen; length of animal 250 μ, greatest width about 60 μ, since it was but partly contracted.

3. A third empty case has sac 125 μ along shorter dorsal side and tube 125 long. Depth of sac 65 and tube width 37, width of flaring lips 50 μ. The axis of the tube rises about 135 degrees away from the axis of sac.

4. Another empty sac has the same length, but depth of only 40 μ, and the tube was but just begun or else broken off, with diameter of about 35 μ.

5. Another specimen has sac 150 long with great width of 80 μ, short tube 88 long and 40 wide with lips 67; it was seated in the branching angle of the hydroid and faced toward base of hydroid.

The contained animal was much contracted into sac, 150 by 60, with nucleus elongated 15 by 25, with lobes 37 by 14 μ, and the left lobe terminated in a papilla $2\frac{1}{2}$ by 10 μ, recalling the *Freia stylifer* of Wright that was stated by Ryder to be probably but a variety and which represents a temporary state as we see it in Folliculinas in the Chesapeake Bay.

6. The sixth specimen measured: sac 125 by 80, tube 150 by 40 with lips 55; inhabited by animal remarkably well expanded, having main body 166 by 40 with nucleus 17 by 22 and lobes stretched out to 92 μ and 10 to 12 wide. This animal contained a large diatom as food, while some of the others contained masses of detritus as if bacteria in digestion. Within the sac next the animal are several nucleated masses, either foreign protozoa or possibly

fragments of disintegrated *Folliculina* arising from division and dying.

Comparing these measurements with those given by Dons for *Semifolliculina boecki,* we see that they are somewhat smaller except for the length of tube; but in the present state of ignorance of limits of species it would be folly to separate this from the *Semifolliculina boecki,* which includes the so similar *Lagotia viridis* of Wright, which Dons has separated from the simpler *Folliculina ampulla* of Mueller.

Not making a new species of the Woods Hole form, we may tentatively refer it to *Semifolliculina boecki* as exhibited in the above evidence; moreover, the form from the adjacent region of Newport described by Leidy, though much like some of Dons's smaller *S. gigantea,* may be the same as *S. boecki,* and we thus have probably this one species along the New England coast, together with the narrow-necked form *Folliculina spirorbis* as quoted by Dons.

Yet along with the above six specimens is one that is aberrant. Its sac and tube in one line stand out freely from the hydroid, attached only at the base. The tube enlarges where joining the sac and envelopes it as a swelling within which the edges of the sac end as free lip or inturned shelf, producing the appearance of a circular valve standing inward from the wall. This empty case is thus much like the *Folliculina telesto* of Laachmann as figured by Dons from Dröbak, page 88, Fig. 2, and later called *Parafolliculina violacea* by Dons.

The dimensions of this single empty case are: length, 250; greatest width, 63; length of tube, 82; width of tube beneath collar, 30; width of collar, or lips, 42; width of swollen tube where embracing mouth of sac, 51; width of sac just below this swelling of tube, 40; diagonal width of inner projecting flange that might be called a valve, 7½. Compared with Laachmann's specimens 200–260 by 60 and with Dons's 260–310 by 55–90, it closely resembles the former in size and the latter in appearance and pro-

portions, as seen in the above Fig. 2. This single specimen agrees very well in size with the six others on same material, but differs in the narrow lips and the swollen base of tube embracing the sac, as well as in the attachment of a sac by end only.

Knowing the normal mode of secretion of sac and of tube, one is tempted to suppose that a single exceptional specimen like this may have arisen by some fault in attachment of a swimmer followed by lack of proper rhythm of secretion, so that sac was attached by end and not along whole side, and that later the animal abnormally started to secrete a second collar within the old junction of sac and tube, bulging out the tube while still soft by pressure of its persisting mushroom and making an inner rim or shelf to represent an imperfect collar after it had already made an insufficient one at the mouth of the abnormally narrow tube. The whole structure would thus be an abnormal product resulting from slight abnormalities in secretional activities of the animal after unusual attitude in attachment. Such an hypothesis for explaining the telesto shape might be extended to all the telesto forms seen hitherto by Laachmann and by Dons, and these are significantly few; thus Laachmann found but *one* specimen on material from Sumatra and Dons found 20 after much search on Eudendrium from the Adriatic and but about a dozen amidst very many *F. ampulla* (*i.e., S. boecki*) from Dröbak, and none at all from North Norway. A form occurring but rarely and found in Sumatra, West Australia, Norway and Woods Hole, Mass., may well prove to be but an abnormality rather than a real species.

The same suspicion of abnormality attaches to four additional sacs found empty and clustered together on the above hydroid material from Woods Hole. Apparently unfinished, they are characterized by great breadth and shortness and by narrow openings where the tube had not yet been added. With length of 110–113 μ, these sacs were 90 μ wide. They thus recall proportions of

Mueller's Folliculina, but the form is evidently so different in each of the four that they may be thought of as greatly shortened *Semifolliculina boecki* in which a premature change of axis in secreting has made the sac very deep where passing over into the tube, which is then expressed as part of the sac and left unfinished. The whole is like a short club foot with swollen ankle.

For the present all the known normal material from the coast of New England (except that referred to by Dons as *S. planorbis*) may be regarded as belonging to those forms described by the name *Semifolliculina boecki* and closely akin to the original *Folliculina ampulla* of most authors.

Turning now to the southern coast, the bottle animalcules first seen by Ryder in the Chesapeake were referred by him to *Freia producta* of Strethill Wright, September 3, 1880. This animal 1,000 μ long extended and 100 when contracted has a dwelling compared to a stocking with spiral ribbon of four to twenty-four turns to the right. Found in vast numbers on oyster shell with Bryozoa, its occurrence agrees with that later reported up the bay by the present author. What is evidently the same form has been seen by me in the Severn and other parts of the Chesapeake Bay in 1912–15 and described without specific identification.

In Dons's classification these Chesapeake forms are evidently *Semifolliculina* and might be included in the widely variant *S. boecki,* but that the tube is so much longer and the collar so relatively narrow.

Moreover, it has the gregarious habit described by Wright, the free-swimmers being stuck together in a secreted "colletoderm," and may be identified as the same as his *Freia* (*Lagotia*) *producta,* if we grant that in his sketch of the animal and bottle he overemphasized the "immensely prolonged" tube in proportion to the sac, which he figures as relatively too short for his comparison to a jack boot.

If, then, we may retain the name producta as signify-

ing a long spiral tube with narrow collar, we will separate it from the other four species *S. gigantea, S. planorbis, S. similis* and *S. boecki* recognized by Dons and may add the characteristic feature of the animal that its nucleus is generally moniliform and the lobes very long and ligulate so as to be compared to the ears of a hare in length by Wright and from their curvature of surface to blades of obstetrical forceps by Ryder—far removed from the funnel appearance of many of the smaller and earlier forms that had been described.

Tubes that have had second additions added to them, described by Wright, were also sometimes observed both by Ryder and by Andrews.

While the ordinary form of the Chesapeake is thus a much longer tube and markedly spiral as compared to the simple New England form, so that it may be provisionally regarded as specifically distinct and referred to *Semifolliculina producta,* there are also other forms of bottles occurring sparingly with the long tubes that are more simple and short than the New England forms, though they may have an added complexity regarded by Dons as a sort of valve and relegating them to the genus *Parafolliculina.*

Of the two species recognized by Dons the Chesapeake form is evidently *Parafolliculina amphora.* Characterized by the wide flat sac attached along its lower face to substratum and joined to short tube which swells out around mouth of sac and then rapidly diminishes to end with upward turn and narrow mouth with little or no collar. The whole enveloped for the most part in a halo of soft secretion and the junction of sac and tube characterized in many specimens by an internal valve-like set of membranes or modifications of the edges of the sac where jutting into the swollen tube. The animal is simple with single nucleus and short arms or funnel and nearly colorless.

The measurements given by Dons for specimens from Norway and Iceland are: length over all 110 to 150, of

which sac is 73, 82, 100, 112, when tube is 27, 27, 50, 37. Width 90–120, narrowest width 40–70. Diameter of swelling of tube 50–80, width of tube about 30–50. Diameter of nucleus from 15–32.

The Chesapeake forms have a much flattened sac, some 130–140 long by 195–110 wide, but only 30 deep. The exact point of passing to tube is various; the tube may be regarded as 25–55 wide and swelling to 57 wide, though it may be but 25 at actual mouth, which rarely has a collar, but may have a flaring of 6 μ. The length of tube may be 30, making the length over all 175. The tube may turn upward from attachment nearly 50 μ when sac is but 30 deep. The nucleus is from 15 by 17 to 15 by 27.

The halo of secretion about sac is some 7 μ or more in thickness. Characteristic of some specimens is the valve, so-called, relied upon by Dons as of generic value. This appears as a dorsal and ventral flap of membrane within the sac at its continuation as the tube and projects forward. These two membranes converge to meet below the center of the cavity, which they close off more or less completely. They vary in number and position and often seem to be lacking.

A description of these amphora forms of the Chesapeake will be published elsewhere and we will here merely add a summary of the foregoing consideration of the probable position of the known American folliculinas in the tentative scheme of classification of the family.

Following Dons we may separate the Folliculinas from the Stentors on account of the more or less marked development of the body as lateral lobes which make a funnel leading toward the mouth and which may be regarded as a specialization of the more primitive feeding apparatus found in *Stentor*.

The Folliculinidæ are thus Heterotrichia with spindle-shaped body prolonged as lateral lobes to form a funnel leading toward the mouth and marked ability to secrete dwellings composed of a sac-like part more or less prolonged as a tube which may reach great length and exhibit spiral structure.

The sac is fastened by secretion to some foreign body and the animal lives sedentary till such times as it may break loose from attachment of foot end to base of inside of sac and then swim free to soon secrete another sac and tube. In free-swimming individuals, lobes and mouth may be absent and later reconstructed. Contractile vacuole absent.

The nucleus is round, oval or moniliform. The micronuclei may be many and minute. Cross division results in halves, of which one may remain and the other escape from the dwelling; the size differs much in different individuals. Complete life cycle, when known, may show that some of the apparently specific forms are but stages in life cycle of others.

Species based so largely upon the forms of the secreted dwellings may eventually prove to be but results of diverse secretional activities within one species.

Conjugation unknown; reproduction by transverse fission follows nuclear condensation and dedifferentiation of peristome; the posterior half grows a new mouth and peristome and soon occupies the old dwelling, while the anterior half swims free with no mouth and simple spiral membranelle zone, secretes a new dwelling, and differentiates new mouth and peristomal apparatus.

The old genus *Folliculina* may be conveniently divided into tentative subdivisions as suggested by Dons; based chiefly upon shape and proportion of the secreted cases.

These groups may be spoken of as genera, namely, *Folliculina, Semifolliculina, Parafolliculina* and *Pseudofolliculina.*

In *Folliculina* Lamarck, as restricted by Dons, the sac is commonly as wide as long, the tube is short and without collar and there is no spiral nor valves.

The only species are the original *F. ampulla* of Mueller and the *F. paguri,* which was Giard's *Pebrilla paguri,* and seems to be an abnormality. The fresh-water *F. boltoni* seems to be the same as *F. ampulla,* and is recorded from England, Switzerland and from France (as the *Ascobius* of Henneguy).

In *Semifolliculina* the sac is longer and the tube may

be very long, with a collar and more or less spiral marking, but no valves.

Some six species may be recognized. The widespread *S. boecki,* being separated by Dons from the old *F. ampulla* and bearing the specific name of Claparede's *Cothurnia,* includes the *Freia ampulla* and *Freia aculeata* of Claparede (which are only stages of growth transformations), as well as the *Lagotia viridis, L. atropurpurea* and *L. hyalina* of Wright. Similar but much more evolved in specialization of tube is the *S. producta* of Wright.

S. gigantea of Dons is the same as Laachmann's antarctic *F. ampulla* and as Stein's *F. ampulla.*

The *S. elegans* of Claparede may belong here as tentative species with mouth of tube incised on one side, but this feature may well be accidental form.

S. spirorbis of Dons is well marked by absurdly narrow tube and is a very minute form that suggests depauperization.

S. similis recently described by Dons from south polar material possesses a very wide tube without spiral. The specimen figured shows tentacular projections of lobe such as we see in transformation stages.

Parafolliculina has a short tube which is swollen just above a narrow connection with the sac and may present internally membranes regarded as valves. There are two known species. The typical species is *P. amphora,* one of the smallest of the bottle animalcules found by Dons to remain the year through in some localities in Norway, while also known from Iceland and, we find, in the Chesapeake Bay. The other species, *P. violacea,* has the case attached only at the base of the sac and not along its entire ventral face as in all above-named species. If this feature is incident to some unusual behavior of the free-swimming stage when about to settle down and construct the sac, the two species may prove to be but one. It was found on the French coast by Giard and is known from south Norway, the Adriatic and West Australia.

Pseudofolliculina has no enlargement of the tube, but,

in fact, the tube and sac grade into one another and stand straight up from the attachment. This is by means of a long cylinder of cementing material, bringing the base of the animal well above the substratum. There may be a simple membraneous valve.

Of the two species, *P. mellita* was taken in the Antarctic in 1902–3 by Laachmann at depths of 350–385 meters and its case has a length .6–.7 mm., but this great length is partly due to the mode of attachment of the sac by means of a stalk of secretion. As this stalk is hollow and filled by a tenuous prolongation of the body of the animal, it may be that this genus also is founded upon individual idiosyncrasies of secretional activity.

The other species, *P. arctica,* has been formed by Dons to include the smaller but similar forms he found in Norway and finally separated from *P. mellita* as smaller, with narrower stalk apparently not perforated.

As the above eleven species have been most studied in England, Germany and Norway, it is natural that Folliculinas are known chiefly from those coasts; yet the known distribution of Folliculinas has already been extended to the Antarctic, the Mediterranean, the White Sea, as well as to the east and west coasts of the North Atlantic. On the east coast of the United States we seem to have four or five species in two of Dons's subdivisions of the old genus *Folliculina,* namely: the most specialized and best known *Semifolliculina producta* of the shores of the Chesapeake; the accompanying small and simpler form *Parafolliculina amphora;* the less well-marked form *Semifolliculina boecki,* first found at Newport, R. I., and later at Woods Hole, Mass.; and finally, as recorded by Dons, from material from the North American Atlantic coast the *Semifolliculina spirorbis;* and if Dons's *Parafolliculina violacea* be a real species, it also is to be credited to Woods Hole.

That some or all of the smaller and simpler forms arise from larger and more complex forms under changing conditions of nutrition in the successive phases of sedentary and free life is a tempting working hypothesis.

Whether these widely distributed marine protozoa which in some cases are able to live in fresh water may not be found in all parts of the world remains to be found out; with attention turned to their discovery, it may be hoped that knowledge of both the life histories and the taxonomy may soon be placed upon a firmer basis.

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SHORTER ARTICLES AND DISCUSSION

THE DUALITY OF EGG-SECRETION

THERE are two properties of egg-secretion which have attracted particular attention. One of these is the power of agglutinating spermatozoa (Lillie[1]); the other is the power of activating the egg (auto-parthenogenesis, Glaser[2]). These effects have been studied separately, but under normal conditions both must bear, in close affiliation, upon the events of fertilization.

I

We are still in the stage of hypothesis. We know that without exudates fertilization does not take place. This was first demonstrated by F. R. Lillie (*loc. cit.*), who removed the exudates. These, as I found later, can be rendered ineffective without removal, by the presence of charcoal.[3] In neither case is fertilization possible. We also know, from the investigations of Lillie, that the agglutinating agent can be neutralized in two ways: either by spermatozoa, or, by some derivative from the egg itself. Inasmuch as fertilization is prevented by this derivative, Lillie has called it "anti-fertilizin."

Lillie also discovered that the blood of the sea-urchin (*Arbacia*) has preventative effects, but these are different from those of "anti-fertilizin." There is in this case no interference with the agglutination reaction; yet the egg remains unactivated.

Miss Woodward[4] and I (*loc. cit.*) have extended the list of inhibitors to include such diverse things as the pigmented substances derived from testicular tissues; fatty and aqueous extracts of the eggs; and now, oleic acid and olive oil. However I shall consider further only the inhibitors discovered by Lillie.

II

These, together with the fact that the exudates are necessary in normal fertilization, are the foundations of the fertilizin

[1] *Science*, Vol. 38, pp. 524–528.

[2] *Biological Bulletin*, Vol. 26, pp. 387–409.

[3] The use of charcoal was suggested to me by Dr. G. H. A. Clowes.

[4] *Journal of Experimental Zoology*, Vol. 26, pp. 459–501.

hypothesis. According to this view, egg-secretion contains a body with two bonds; one normally unites with a sperm-borne valence; the other, with a valence borne by the egg. Agglutination is a symptom of the first union; activation of the egg, a symptom of the second. "Anti-fertilizin" occupies the agglutinating valence and hence the normal union of sperm and fertilizin is rendered impossible. The inhibitor in the blood, on the other hand, is effective because it binds the second valence of the fertilizin which in consequence cannot unite with its normal receptor in the egg. In the language of Ehrlich, fertilizin is an amboceptor with ovophile and spermophile side-chains; and, normal fertilization involves the formation of a chemical compound which, written in linear fashion, can be thought of as sperm-receptor—spermophile side-chain-ovophile side-chain—egg-receptor.

The keystone of the hypothesis is the amboceptor. It symbolyzes the effects of the secretion on spermatozoa and the effects on eggs; it symbolizes also the information gotten from the two inhibitors—the one that prevents agglutination and fertilization and the other which prevents merely fertilization. The amboceptor stands vaguely for the recognized duality of the secretion. The question is, can we precipitate the amboceptor from the realm of the symbolic and bring it within the sphere of the commoner conceptions of physics and chemistry?

III

First of all, are we compelled to think in terms of the amboceptor? The alternative, of course, is a two-body view. Lillie's book,[5] (p. 231) decides against this because there is "a parallel between absence or loss of agglutinating substance and the capacity of the egg for being activated. The same results would be attained if there were two substances concerned . . . but since the two effects . . . appear and disappear together . . . the writer assumed that they may be regarded as due to a single complex substance." Again (p. 232), in discussing the effects of the inhibitors, Lillie writes:

> This still does not prove that sperm agglutination and egg activation are due to the action of a single substance, but it shows again by a different method that the capacity for producing both effects is present

[5] "Problems of Fertilization," University of Chicago Press, 1919.

simultaneously in the egg secretion and the assumption of a single substance is the simplest hypothesis.

A much stronger argument for the one-body view can be advanced. "Anti-fertilizin," by hypothesis, prevents normal fertilization by occupying the spermophile side-chain; yet, "anti-fertilizin" also prevents auto-parthenogenesis (Woodward). This, we might suppose, would indicate a relationship between the two side-chains, such that removal from the reaction system of the one also results in the removal of the other. This state of affairs is actually realized under certain conditions. For example, I have been able to isolate from charcoal materials with agglutinating and activating properties. However, if we infer from these observations, a single body, the reaction of "anti-fertilizin" with fertilizin can hardly serve as evidence that the latter has two side-chains.

IV

In 1918, Miss Woodward[4] reported experiments that bear intimately on the problem. By saturating *Arbacia* secretion with $(NH_4)_2SO_4$ she secured a white flocculent precipitate which after purification by dialysis proved to have very intense agglutinative powers but no capacity for initiating the development of the egg. She called this precipitate, agglutinin.

An initiatory agent, also, was precipitated. $BaCl_2$ was added to the secretion and after removal of the sea-salts, the primary deposit was treated with $N/10$ HCl. As soon as the acid had been freed from $BaCl_2$, acetone was used in excess to bring down a second precipitate, heavy and flocculent. This, after purification with absolute alcohol and ether, dried as a white powder, soluble in both sea-water and distilled.

This second precipitate had marked parthenogenetic effects, but no power to agglutinate sperm. Miss Woodward called the substance, lipolysin, a name which, as I shall show elsewhere, is justified since the material accelerates the hydrolysis of fats.

V

What bearing have these precipitations on the issue? According to Lillie's book, p. 240, "separation under the conditions of chemical analysis may possibly denote a splitting of a single substance of the normal egg."

A. The Ammonium-Barium Precipitations

1. The agglutinin can be salted out with $(NH_4)_2SO_4$; the lipolysin can be brought down with $BaCl_2$. If we divide a given exudate into two portions, we can precipitate in one, first the agglutinin and later the lipolysin, whereas with the other fraction we can proceed in a manner exactly the reverse.

2. Precipitation in the two cases differs inasmuch as the effective concentration of the $BaCl_2$ is $N/7.5$; that of the $(NH_4)_2SO_4$ in the neighborhood of $5N$.

If precipitation in the two instances is uncomplicated by chemical unions between precipitate and the reagents used to produce them, the case is decisive for the two-body view since it is unlikely that a molecule can be split chemically, merely because one part has solubilities different from those of another part. However, the case is probably not so simple. Very possibly we are dealing with ammonium agglutinate and barium lipolysinate. If this is true, then all we can say is that the amboceptor, present by hypothesis at the outset, breaks down between the agglutinating and the activating valence no matter which of the two groups is bound first.

B. The Charcoal Method

Charcoal removes practically the entire organic reaction system. This can be recovered in its essential parts, by subsequently treating the charcoal with $N/10$ HCl. From the clear solution so gotten acetone throws down a voluminous precipitate in two well-marked stages: the first fraction, without agglutinating powers, is lipolytic; the second strongly agglutinates the sperm.[6]

Very likely factors are involved in precipitation by charcoal which are not present when $(NH_4)_2SO_4$ and $BaCl_2$ are used. Very possibly the amboceptor is split by the charcoal; or, it may not be split until the acetone in the HCl reaches a certain concentration. In any case the cleavage of the molecule gives results identical with those gotten by Miss Woodward's methods.

C. Reactions to Heat

Lillie has shown that the agglutinating material is extremely resistant to heat. Exudate which has been boiled agglutinates spermatozoa perfectly well. It has, however, lost its capacity as a parthenogenetic agent (Woodward).

[6] It is very important to guard against impurities in the charcoal. These after extraction with HCl give a voluminous precipitate with acetone.

On the one-body view we must reckon with the following possibilities: (1) a rupture of the molecule; (2) the destruction or alteration of the ovophile side-chain.

VI

Looked at conservatively, the methods of precipitation alone do not enable us to decide the one-body–two-body issue. The same thing may be said of the results of boiling. One conclusion, however, is certainly warranted: there is a constitutional weakness in the amboceptor so pronounced that this molecule breaks down with the greatest ease and, under very diverse conditions, always cleaves in a manner that separates the spermophile from the ovophile side-chain. Indeed one doubts whether the amboceptor can hold its ovophile side-chain after the agglutinating group has united with the receptors of the sperm. This, if true, would be awkward for the theory.

VII

D. FILTERABILITY

Lillie has shown that exudates which have passed through Berkefeld filters no longer agglutinate spermatozoa. The agglutinating material, in this case, can be recovered, as Miss Sampson found last summer, by washing the filter-cone in sea-water. In the original filtrate I was able to demonstrate lipolysin.

It is conceivable that the agglutinating material is not filterable because of "chemical adsorption." If this is true, then filtration becomes merely another method for the chemical decomposition of the amboceptor. Yet, we can account in this manner for only a portion of the agglutinin held back. It seems very unlikely that the fraction which can be recovered by merely washing the filter-cone, was held chemically bound. Moreover, on account of the metal band which holds the filter-cone in position, a remnant of the secretion invariably fails to pass through. This remnant has a higher agglutinating value than the original exudate. It seems safe to conclude that the agglutinating material is held back mechanically. If correct, these considerations based on filtration are conclusive, for we know of no cases in which a substance is chemically decomposed merely because the whole molecule is unable to get through the pores of a filter.

The results of filtration seem to me to necessitate the two-

body view. None of the facts explicable by the amboceptor appear to become inexplicable when this body is analyzed into an agglutinin and a lipolysin. As for the difficulties of constant association, these are no greater in this case than the difficulties which arise because serum albumin and serum globulin always occur together in the blood.

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February 7, 1921

DESCRIPTION OF A PECULIAR YOLK MASS IN THE OVIDUCT OF A HEN

A DESCRIPTION of this specimen seems desirable for two chief reasons: First, because of its unique nature; second, because it supplies the data with which to answer the question whether reverse movement, possibly antiperistalsis, occurs in the formation of double eggs and similar anomalies.

The specimen was presented to the histological laboratory by Ashton Barbour, of Charlottesville, Va., six hours after it had been removed from an apparently normal year-old hen. He described it as having been taken from the "egg-bag." When questioned, he was positive that he had noticed a number of developing eggs, "little yellow balls," attached to the dorsal surface of the abdominal cavity. His anatomical observations stopped at this point.

The specimen was roughly egg-shaped, and of a yolk or yellowish-orange color. Between ends it measured 9½ cm. Its diameter at the point of greatest width (about one third the distance from the wider end) was 8 cm. When opened the mass was found to contain an egg of average size, with a shell of normal hardness and thickness. The egg was not exactly in the center, but was placed slightly to one side and towards the larger end, causing a variation in the thickness of the lateral walls of the enveloping mass (Fig. 1). At the thickest point the lateral wall was 2 cm. thick, at the thinnest point 1 cm. At the larger end the wall measured ¾ cm. in thickness, at the smaller end it measured 1½ cm. in thickness. The weight of the enveloping portion of this yolk mass, after the enclosed egg had been removed, was about 190 gms. The general ovoid shape of the mass was presumably determined by the enclosed egg.

The mass was made up of layers of yellow, yolk-like material

between which were scattered irregular laminæ of a glairy, mucus-like substance (Fig. 2). In places these laminæ had apparently hardened to form clear, firm, gelatinous areas. The outermost yellow layer was about 1½ mm. thick, and completely encircled the mass.

On one side, at the point of greatest diameter, there was a

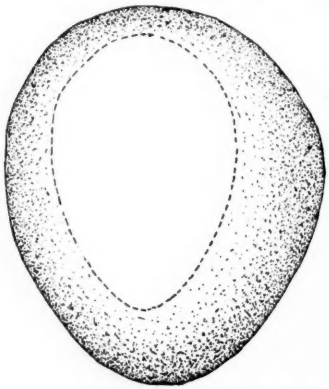

FIG. 1.

FIG. 2.

FIG. 1. Diagram of yolk mass from oviduct of hen. The inner broken line represents the outline of the inclosed normal egg. The peripheral stippled area represents the laminated envelope of yolk and albumen. Natural size.

FIG. 2. Diagram of transverse section of the yolk mass. The central circular area represents the inclosed normal egg. The peripheral stippled lamellæ represent layers of yolk, the clear lamellæ, layers of albumen. Natural size.

shallow depression, about 2 mm. deep, almost the size of a dime in circumference. This was due to a thinning of the two external enveloping layers at that point. A dark, reddish discoloration partly surrounded this depression in the form of a crescent. There were a number of small granule-like hillocks, about the size of a pinhead, on the surface of the smaller end. These elevations probably represent casts of the mouths of the oviducal glands, produced under pressure of the enlarging mass against the constricted confines of the oviducal walls.

When the egg which the mass inclosed was removed and opened, it was found to be filled with a yellowish liquid, in which there were bits of a translucent and whitish mucus-like substance, the remains most probably of the disintegrated chalazæ. The odor of this liquid was not offensive. It may be best described as musty.

Portions of the yolk mass were imbedded in celloidin, sec-

tioned, and the sections stained with hematoxylin and eosin. Transverse sections through this laminated cortical material revealed layers of yolk granules and spherules intermingled with layers of clear, hardened, egg-white. There were no indications of the presence of shell, or any unequivocal evidence of shell membrane, in any of the sections.

Abnormal eggs have been observed and discussed by biologists for many years. In the AMERICAN NATURALIST for January, 1906, G. H. Parker (4) has treated the subject of double hens'-eggs, "ovum in ovo," very fully. He reviews much of the previous literature on the subject and describes several specimens of his own, similar to a specimen of a large, double egg which belongs to the laboratory of histology here. Parker supports the theory of Davaine (2) and others concerning the formation of double eggs.

Briefly the theory is this: The egg is moved by peristalsis from the ovary to the distal end of the oviduct. As it passes down the oviduct it receives the usual coverings of albumen, shell membrane, and shell. The egg is now a normal egg, ready to be laid. But for some reason, instead of the egg being laid normally, antiperistalsis occurs and the egg is carried back up the oviduct. In the upper portion of the duct it meets another developing egg coming down. The two pass down together. Albumen is laid on and a common shell covers the whole mass. We now have a giant egg, approximating the size of an ostrich egg, which contains a second complete normal egg along with its own yolk and albumen.

Curtis (1) has described a number of interesting anomalies in hens' eggs, including double eggs and other anomalous specimens, either with a membrane only or with both shell and membrane. She reports finding eggs in the body cavity of fowls whose oviduct had been ligated in the isthmus, or shell gland. She does not venture to commit herself, however, as to whether antiperistalsis is the means by which the egg is carried back up the duct. Patterson (5) describes a specimen which has two shell membranes. He explains this condition on the assumption that antiperistalsis had occurred twice before the egg was laid. Hargitt (3) describes an interesting gourd-shaped egg. None of these authors, however, mention an anomaly similar to our specimen.

What may be assumed to have happened in the formation of

our specimen was this: The first egg which left the ovary of the young hen passed down the oviduct normally and had albumen and shell laid on in the usual manner. The egg passed on into the lower part of the uterus (shell gland), but, due to injury, congenital occlusion of the vagina, or some obstruction, the egg could not be laid. Such interference with normal oviposition, either congenital or acquired, stimulated a reversed movement (probably antiperistalsis) and the normal egg was carried back up into the oviduct and lodged there. More eggs left the ovary, took on albumen as they passed down the duct, but coming into contact with the preceding egg which occluded the duct, where broken by pressure, and the soft yolk and albumen collected about the obstructing egg. In this way the yolk mass about the egg acquired its large dimensions.

We can thus locate very closely the exact position of the anomalous yolk mass in the oviduct of the hen. Since it contained layers of albumen it must have lodged below, or in the lower part of the portion of the oviduct where albumen is laid onto the yolk; and since there was no shell whatever within the cortex of the mass, it must have lodged above the point in the oviduct where shell is formed. Again, antiperistalsis, or at least reversal of normal movement, must have occurred because the inclosed egg comprised a shell, and so must itself have gone the full length of the oviduct into the uterus, while at the time the enveloping yolk mass was formed the original egg must have been above the shell-forming level.

The above evidence, combined with the evidence of Davaine (cited by Parker) and Curtis, who report finding soft-shelled eggs in the body cavity of fowls, seems to prove conclusively that something of the nature of antiperistalsis in the oviduct does occur. The inference seems warranted with regard to our specimen, that if the included normal egg could have retraced its course down the oviduct in company with the next following egg, instead of lodging permanently in the preuterine portion of the oviduct, a common shell would have been laid onto the two eggs. This shell would have included the two together, and the result would have been an ordinary "ovum in ovo," similar to the ones described by Davaine, Parker, Patterson, Hargitt, Curtis and many others.

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THE HEREDITY OF ORANGE EYE COLOR IN DROSOPHILA MELANOGASTER[1]

THERE are three points of special interest in the heredity of orange eye color. First, the eye color is due to the presence of two sex-linked genes; second, these two genes may separate in the F_1 female when orange is crossed to the wild stock, producing in F_2, in addition to orange and wild type males, a third eye color called salmon[2]; third, when an orange male is crossed to the parent stock, reduced, only orange and wild type males appear in F_2.

Orange first appeared in the sixth generation of the plus selected line of the mutant strain reduced. Eleven males appeared from a single pair of parents. Several of these males (orange reduced) were mated to wild type females. All F_1 flies had red eyes. Twenty-seven F_1 pairs were mated (Table I). Of the F_2 males 850 were wild type (red-eyed), 785 were orange reduced, 585 were reduced, 586 salmon, 15 orange, and 6 salmon reduced. This behavior led us to suspect that orange

[1] Contribution No. 182.

[2] Since this paper went to press Professor Morgan kindly sent us some stock of garnet. The crosses show salmon and garnet to be the same.

TABLE I

F_1 ♀ WILD TYPE × F_1 ♂ WILD TYPE (FROM ORANGE REDUCED ♂ × WILD TYPE ♀)

Bottle Number	Female	Male					
	Wild Type	Wild Type	Reduced	Orange Reduced	Salmon	Orange	Salmon
789....	194	65	42	61	46		
798....	127	31	22	33	34		
804....	92	15	19	30	11		
808....	109	26	24	29	30	2	
814....	72	17	9	14	13		
815....	140	33	23	30	16	2	
816....	123	22	16	25	14		
831....	65	17	8	12	7		
854....	77	30	11	14	9		
859....	69	18	11	13	15	1	
860....	66	18	13	22	13		
1,431....	100	30	13	25	17	1	
1,372....	174	48	39	31	31		
1,494....	28	12	7	8	5		
1,495....	173	45	34	37	30	1	
1,557....	149	31	25	42	22	1	
1,569....	112	87	25	29	19	2	2
1,570....	106	20	20	17	17	1	
1,571....	66	22	9	10	15		
1,674....	111	26	27	27	33		
1,751....	175	62	41	55	42	1	1
1,736....	207	64	27	72	26		
1,724....	174	55	21	36	24	1	
1,720....	158	35	30	40	34	1	
1,441....	111	39	27	32	21		1
2,431....	74	22	20	22	19	1	
2,433....	81	20	22	19	23		2
Totals...	3,133	850	585	785	586	15	6

TABLE II

$F_1 \times F_1$ [FROM ♂ SALMON × ♀ WILD TYPE (FROM STOCK)]

Bottle Number	Female	Male	
	Wild Type	Wild Type	Salmon
1,612.....	146	65	54
1,665.....	123	51	34
1,668.....	175	69	68
1,679.....	121	69	54
1,682.....	130	68	54
1,689.....	161	63	56
1,701.....	117	54	62
Totals...	963	439	382

eye color was due to the presence of two sex-linked genes, one of which by itself produced no visible effect, but when the two were brought together orange was the result. With this hypothesis in mind we attempted to prove or disprove it and believe we have demonstrated that the suggested explanation is the correct one.

First, some of the F_2 salmon males were mated to wild and the eye color shown to be due to a single sex-linked gene. An examination of the F_2 males when an orange reduced male is mated to a wild female (Table I), shows that practically all salmon males (586 out of 592) are wild type with respect to bristle number, and that practically all orange males (585 out of 600) are reduced. These results showed that the modifying gene which changes salmon into orange is closely linked to reduced. Most of the F_2 reduced red-eyed males then should carry this modifier and we should be able to produce orange flies in the second generation by mating these reduced F_2 males to salmon females. Such crosses have been made and the results show that the F_2 reduced males do carry such a gene which when added to salmon produces orange. The two genes for salmon and salmon modifier are brought together in the same chromosome by crossing over in the F_1 female (Table III).

TABLE III

(*a*) Reduced ♂ (F_2 from Orange Reduced ♂ × Wild ♀) × Salmon ♀

Wild type ♀ ..... Salmon ♂
966 823

(*b*) F_1♀ [from (*a*)] × Orange ♂ from Stock

Bottle Number	Female			Male		
	Wild Type	Salmon	Orange	Wild Type	Salmon	Orange
260.....	233	66	134	248	117	116
261.....	102	49	62	85	44	47
262.....	157	52	94	184	74	61
263.....	69	23	38	63	42	29
266.....	45	20	34	54	23	24
Totals ..	606	210	362	634	300	277

That the modifying gene lies in the **X** chromosome rather than in one of the autosomes is clearly shown by the F_2 ratios when orange is mated to a wild female. If the gene were reces-

TABLE IV

F_1 ♀ WILD TYPE × F_1 ♂ REDUCED (FROM SALMON ♂ × REDUCED ♀)

Bottle Number	Female		Male					
	Wild Type	Reduced	Wild Type	Reduced	Salmon	Salmon Reduced	Orange Reduced	Orange
8,347....	133	112	60	76	72	4	43	1
8,348....	124	119	46	57	67		40	3
8,354....	88	105	47	43	80	4	47	
Totals ..	345	336	153	176	219	8	130	4

sive and in an autosome all the F_2 females should be wild type. Such is the case. Of the F_2 males, four should be red-eyed, three salmon, and one orange. If the gene were dominant, the ratios among the males should be four red, one salmon, and three orange. The actual results are given in Table I. We find neither of the former ratios, but 1435 red-eyed, 800 orange, and 592 salmon. The fact that there is a linkage between the genes for salmon modifier and reduced is further evidence that the gene for salmon modifier is in the X chromosome.

Orange eye color, then, is due to two sex-linked genes, one of which when alone produces salmon; the other called salmon modifier, when alone produces no visible effect, but when added to salmon produces orange. The discovery of such a modifying gene is not new as Bridges has demonstrated seven of them which modify eosin eye color. It does lend support, however, to the presence and behavior of such genes.

These same orange reduced males when mated to reduced females give a very different result from the cross to the wild. When crossed to reduced, orange behaves as a single sex-linked character (Table II). Why this difference? At first it was our impression that the reduced strain carried a non-crossover factor. This was disproved, however, by mating reduced to other sex-linked characters. The other alternative and the correct interpretation we think, is that the reduced strain is homozygous for the gene for salmon modifier. This is easily demonstrated by mating salmon to the reduced line. If the reduced line carries the gene for salmon modifier, then a cross of this line to salmon should give some orange males in F_2. Such is the case (Table IV). The reduced strain, then, carried the gene for salmon modifier before the gene for salmon appeared.

No attempt has been made to locate accurately the genes for

reduced, salmon, and salmon modifier. Sufficient crosses have been made, however, to approximate their positions, which are as follows: reduced, 5.24; salmon modifier, 5.94; salmon, 41.33.

Description of Tables

Table I gives the results of mating the F_1 flies produced by crossing an orange reduced male to a wild type female. All the F_2 females are wild type. Of the F_2 males 850 are wild type. 585 reduced, 785 orange reduced, 586 salmon, 15 orange, and 6 salmon reduced.

Table II gives the F_2 results of mating a salmon male to a wild type female. Of the males 439 were wild type and 382 salmon.

Table III (*a*) and (*b*), gives (*a*) the results of crossing an F_2 reduced male (from an orange reduced male $\times$ a wild female) to a salmon female. The females are wild type and the males salmon. In (*b*) the F_1 wild type females were mated to orange males. The orange males were used instead of the salmon to bring out orange in the females. Orange males and females appear. Hence the F_2 reduced males must have carried the gene for salmon modifier.

Table IV gives the F_2 results of crossing a salmon male to a reduced female. Orange males appear. Hence the reduced line must be homozygous for salmon modifier.

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AN APPARATUS FOR MICRODISSECTION

To secure the greatest accuracy in the control of needles for cell dissection an apparatus has been devised on the following plan. The needle is attached to a right angled triangular plate (Fig. *A*), each corner of which is moved by a milled headed screw. The working of any screw causes the movement of the plate about a line through the points of the other two screws as an axis, thus producing motion of the needle point in the three planes of space by manipulation of the screw heads. The nature of the bearings of the screw points on the plate eliminates all side play. The conical end of one screw works into a circular

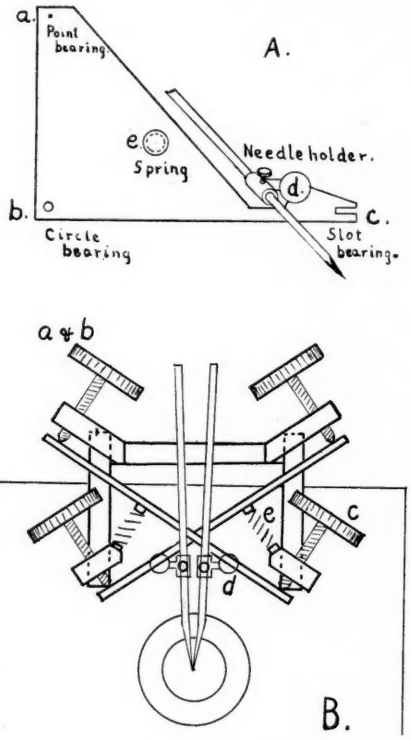

FIG. *A*. Detail of movable plate which bears the needle, illustrating the mechanical principles involved. See text.

FIG. *B*. Diagram of one type of the apparatus clamped to the microscope stage, designed to secure firm needle support close to the microscope focus. The fifth and sixth operating screws are directly beneath two of those figured (*e.g.*, *b* beneath *a*). The vertical movement and one horizontal movement of the needle point are secured by turning one screw each (*a* and *c* respectively); the second horizontal movement, by turning two adjacent screws together (*a* and *b*, with two fingers of one hand). Coarse adjustments are made by moving the needle at the ball-and-socket joint *d*.

hole through a corner of the plate (*b*, Fig. *A*); the conical point of the second screw works into a slit in its corresponding corner (*c*), the third screw point (at *a*) bears on the plane surface of the plate; these constitute what may be termed a cone-slot-point support. The cone-circle bearing prevents side slipping of the plate in any direction; the cone-slot bearing prevents revolution of the plate about the cone-circle bearing. A single spring (*e*)

against the center of the plate holds its bearings firmly against the three screw points, and takes up all lost motion perpendicular to the plate, in the screw threads.

This mechanism has been employed in two styles of dissection apparatus. In one type (Fig. *B*) the aim has been to secure a short needle length, and close proximity of the needle's support to the axis of the microscope lens, with consequent freedom from vibration of the needle and a long leverage in the control of its point. In the other type (Fig. *C*) the apparatus is set away from the microscope axis, to allow the greatest possible freedom of manipulation of objects on the microscope stage. In the former type, the plates bearing the needles cross at right angles, under the microscope nosepiece; in the latter type, they are set beyond the edge of the microscope stage, nearly parallel. Each instrument bears two pipettes or needles operated by three screws apiece. The needles may be attached to the plates either by wax, which when warmed makes possible a coarse adjustment of the needles, or by a universal ball and socket joint, clamped loosely enough to allow gross manipulation under the low power.

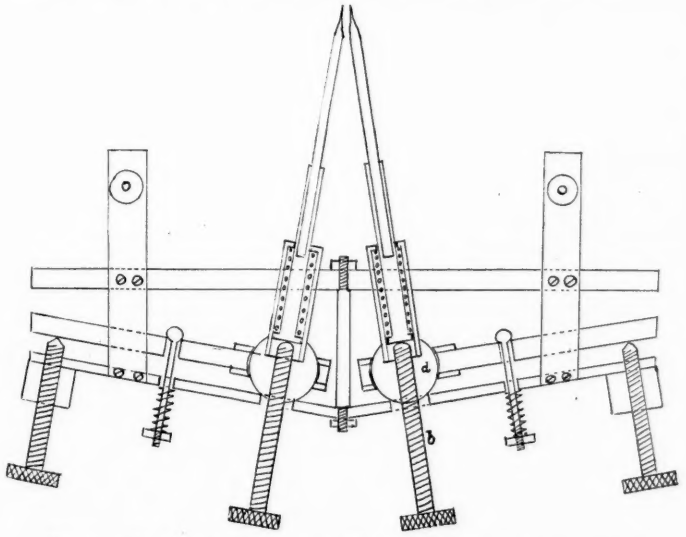

FIG. *C*. Diagram of second type of the apparatus, with parts arranged to leave the microscope stage free for more convenient manipulation of objects upon it. The ball-and-socket joint for coarse adjustment (at *d*) is here made to serve as a pivot in place of the corresponding screw point (*b*); this screw then works through this joint to produce one motion of the needle, thus avoiding the necessity of turning two screws together to secure this movement, as in Fig. *B*.

The advantage of this type of needle control over the sliding motions of the Barber pipette holder lies in greater ease and convenience of manipulation as well as in greater refinement of needle control. The simplicity of the apparatus is such that its manipulation requires no great degree of technical skill, and in its construction have been avoided as far as possible all elaborate and complicated adjustments. The screw heads turn easily enough to be rolled under pressure of one finger each. The complete apparatus for holding two needles may be made to occupy a three-inch cube, and clamped close under the nosepiece; five of its six screws can be operated with one hand, leaving to the other, the mechanical stage ratchets and the sixth needle screw adjacent to these. The needles may be held so close to their points that no vibration is noticeable, especially since no moving part is handled during manipulation. The needle points move in arcs of circles, but since the ratio of length of arc to radius is small, these are virtually straight lines, in the plane of, and perpendicular to the plane of the focus of the objective.

A more detailed account of the working of this instrument, and a description of various devices that may be added to facilitate needle manipulation, will be included in a subsequent paper dealing with the results of its application to the study of protoplasmic activity.

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